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VOLUME XI JULY, 1924 No. 2

OBSERVATIONS ON THE IMPROVEMENT OF POOR PASTURE IN THE WEST RIDING OF YORKSHIRE

BY J. C. LYNN, M.Sc. (AGRIC.),

AND D. ANDERSON LYNN, B.Sc. (LOND.), N.D.A. (HONS.).

(With 6 Text-figures.)

THE district in which the following observations were made comprises the northern portion of the West Riding, and is a tract of country of about 1100 square miles, with Skipton-in-Craven as its approximate centre. It is a hilly area, consisting for the most part of rough pasture and moorland, intersected by the four fertile valleys of the upper reaches of the Nidd, Wharfe, Aire, and Ribble.

The two main types of geological formation are Millstone Grit and Mountain Limestone. The greater portion of the eastern part of the district lies on the former, through which, however, appear numerous outcrops of Limestone, and on which, also, are tracts of River Alluvium and Boulder Clay. Westward and to the north of Skipton the main formation is Mountain Limestone. The bulk of the Boulder Clay being detritus from Millstone Grit, Mountain Limestone and Yoredale beds, the Boulder Clay soils are not so inherently short of lime as are those on Millstone Grit.

The average annual rainfall in Skipton is 38 ins., but much of the rough pasturage on the hills around is subjected to a rainfall considerably heavier.

The principal object in the following studies was to ascertain the most efficient methods of improving the various types of pasture situated in this district (with the exclusion of moorland pasture); and the changes in the nature of the herbage induced by the various methods of treatment. It is not suggested that the conclusions arrived at can be applied indiscriminately elsewhere.

The botanical analyses were made[1] during the summer of 1922. The previous summer was exceptionally dry, and many of the pastures became parched; whereas, with the exception of May, the summer of

[1] After the manner described by Armstrong (*Journ. Agric. Sci.* Dec. 1907).

1922 was exceptionally wet. As so often happens after a dry year, clover was unusually abundant in 1922; and this fact should be borne in mind when the analyses are being read.

The grass-land in the district can be grouped conveniently into three classes, as follows:

I. High-lying rough pasture.
II. Better-class pasture.
III. Moorland pasture.

The present studies are confined to I and II only.

I. HIGH-LYING ROUGH PASTURE.

The type of pasture referred to under this heading is, though not moorland, frequently bounded by moor-pastures growing little but heather and bilberry. Many of the fields are intakes from the moors. Such pasture may be classed under two headings as follows:

(1) White Bent land.
(2) *Agrostis* pasture.

(1) *White Bent land.* There are very large areas of upland pasturage in the West Riding of Yorkshire where the chief constituent of the herbage is White Bent (*Nardus stricta*). Generally speaking, such pastures occur on the Millstone Grit formation, but they are to be found also on gritty drifts of Boulder Clay. With White Bent is invariably associated a mat (or "fogg") which varies in thickness from 2–4 ins. On the better types of White Bent land *Festuca ovina* forms an appreciable proportion of the herbage, and as the percentage of this grass increases, so also does the value of the pasture improve. With *Nardus* is usually associated Heath Bedstraw (*Galium saxatile*), Field Wood Rush (*Luzula campestris*), and Tormentil (*Potentilla tormentilla*). In the damper types of such land various species of Carex establish themselves, and Flying Bent (*Molinia caerulea*) and Stool Bent (*Juncus squarrosus*) become prominent.

It is well known that the formation of mats is due to sour soil-conditions, which interrupt the work of those bacteria which are responsible for the decomposition of dead organic matter. When alkalinity is restored by the application of lime, and the soil once more becomes suited to the activities of such bacteria, the mat is decomposed and absorbed into the soil. Better grasses and clovers have then a chance to compete against the coarser plants, which alone can tolerate a sour and matted condition of the soil. It is interesting to note that in the majority of cases (*vide post*) lime will not only remove the mat and induce a growth

of better grasses, but that it will produce, in addition, a luxuriant growth of Wild White Clover.

The removal of the mat and the better soil-conditions obtained after liming do not in themselves seem to be adequate reasons for the remarkable growth of clover that so frequently results from liming on such soils; and it has been suggested that the mat itself might contain a sensible percentage of phosphates which, on its decomposition, would be liberated in the soil, and become available as plant-food. In November 1921 three turves, each 1 foot square, and having a 3 in. mat, were cut from a rough White Bent pasture, overlying Millstone Grit. These were analysed by the Department of Agricultural Chemistry, University of Leeds, with the following results:

	% N_2	% P_2O_5	% moisture	Loss on ignition	Weight in grams per sq. ft.
Sod A	1·98	·220	8·8	68·46	1050
„ B	1·75	·220	7·53	60·02	1030
„ C	2·09	·216	10·79	68·64	613

It will be seen that such a mat contains an appreciable amount of nitrogen and phosphoric acid. The latter, in fact, on the average analysis of the above three turves, is equivalent to a dressing of 12 cwts. of 30 per cent. basic slag per acre, assuming the phosphates present to be of value equal to those present in slag; and it is reasonable to suppose that, although the available phosphates may form only a small percentage of the total phosphates liberated, they nevertheless have a considerable influence on the growth of the clover which usually follows the decomposition of such mats.

It is to be observed that mats on White Bent land are particularly tough and fibrous, and that there is no admixture of soil with them. The soil under such mats will be found, even after continuous heavy rains, to be quite dry.

A number of cases of the improvement of White Bent land have been studied, three typical examples of which are here cited.

Case A. A rough pasture, 25 acres in area, sloping to the south, and dry. Soil, a sandy loam, overlying Millstone Grit. Altitude, 600 ft. The untreated portion of the field is covered by a mat, 2–3 ins. in thickness, of undecomposed vegetable matter; and there is a lime-requirement (as determined by the Hutchinson-Maclennan method) of 41 cwts. per acre. This area is valued by the tenant at 5*s.* per acre rent.

A portion of the field, 6 acres in area, was limed in 1874, at the rate of about 5 tons per acre of cob lime. This plot stands out conspicuously

as a bright green patch in the distance, and on close inspection it is found that the mat or skin has disappeared and the ground is firm to the tread. It has a lime-requirement of 10 cwts., and is valued by the tenant at 15*s*. per acre rent.

To a strip down the middle of the limed area high-grade slag was applied ten years ago at the rate of 10 cwts. per acre. The effect of the slag on this strip is very striking, the herbage showing up as distinctly greener than the "limed only" area, and the clover being more conspicuous and more vigorous. Stock are continually working on this slagged portion, which is consequently always bare. At the same time a portion of the unlimed area received a similar dressing of slag; but, on examination and analysis in 1922, the herbage on this portion showed no differentiation from that on the untreated part of the field.

In February 1922, 30 per cent. slag was applied at the rate of 10 cwts. per acre to a plot one-tenth of an acre in area on the limed portion, and extending also over the unlimed portion for another one-tenth of an acre. When the plot was visited six months later, in August 1922, the effect of the slag on the limed area was already very marked; and the clover in it even more vigorous than in that slagged ten years ago; whereas on the unlimed area no result could be observed. The tenant values the limed-and-slagged area at 20*s*. per acre rent.

The differences in herbage induced by these various methods of treatment is evident from the accompanying table. For comparative purposes it was decided to group the constituents of the herbage into four classes, as follows:

(1) Leguminosae.
(2) Comparatively useful grasses.
(3) Comparatively useless grasses.
(4) Weeds (and bare space).

It is realised that any division of grasses into classes such as the above can at best be but arbitrary, and a great deal of latitude must be allowed to the terms "Comparatively Useful" and "Comparatively Useless." Although, for instance, *Molinia* is grouped in Class 3, it is none the less one of the most valuable grasses in the summer months on certain types of unimproved hill-land. Compared with *Nardus*, it is a useful grass; compared, however, with the better-class grasses such as the Fescues, it is an inferior grass, and is hence classed as such. After a lengthy consideration and two years' observation of the grazing of the plots, the grasses were grouped as below, and this classification has

been adhered to throughout the series of analyses, so that the diagrams constructed therefrom might have some comparative value.

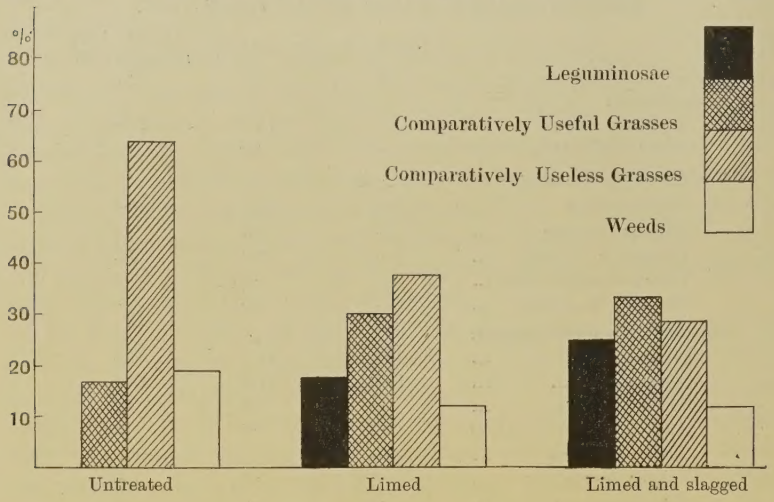
Botanical Analysis of Case A, 2nd August 1922.

Species	Untreated %	Limed 1874 %	Limed 1874 / Slagged 1922 %
Leguminosae:			
Trifolium repens	—	13·5	24·8
Lotus corniculatus	—	4·0	—
Comparatively useful grasses:			
Festuca ovina ...	16·3	25·1	29·4
Phleum pratense ...	—	·8	·4
Cynosurus cristatus ...	—	2·8	3·2
*Poterium sanguisorba ...	—	·8	·2
*Plantago lanceolata ...	—	·5	—
Comparatively useless grasses:			
Nardus stricta ...	45·4	11·7	5·9
Agrostis sp.	10·3	17·9	13·7
Holcus lanatus ...	·4	7·6	6·9
Anthoxanthum odoratum	·3	·2	·3
Molinia caerulea ...	3·5	—	—
Triodia decumbens ...	·9	·3	1·4
Poa compressa ...	·2	—	·3
*Juncus squarrosus ...	2·4	—	—
Weeds:			
Galium saxatile ...	9·4	1·7	—
Potentilla tormentilla ...	5·2	1·4	1·0
Viola sp.	—	·7	·7
Ranunculus sp. ...	—	Trace	·7
Prunella vulgaris ...	—	1·4	1·8
Euphrasia officinalis ...	—	·9	1·0
Veronica sp.	—	Trace	—
Bellis perennis ...	—	·2	·2
Polygala vulgaris ...	·3	Trace	·1
Aira caespitosa ...	Trace	Trace	—
Luzula campestris ...	3·3	3·5	2·3
Carex sp.	—	1·0	2·6
Moss	·5	1·2	1·4
Leguminosae	Nil	17·5	24·8
Comparatively useful grasses	16·3	30·0	33·2
Comparatively useless grasses	63·4	37·7	28·5
Weeds	18·7	12·0	11·8
Totals ...	98·4	97·2	98·3

* For purposes of convenience, throughout the tables, *Poterium*, *Plantago* and *Achillea* have been included in the "Comparatively useful grasses," and *Juncus squarrosus* in the "Comparatively useless grasses."

The results of the analysis may be illustrated diagrammatically as follows:

Diagram to illustrate analysis of Case A.



While the botanical analysis was being made, it was observed that, although liming had not entirely eradicated it, the *Nardus* on the limed and limed-and-slagged areas was interspersed with other plants, its tufts being quite small, much finer, and in many cases grazed almost as closely as the surrounding herbage. Much of the *Nardus* had been pulled out by the roots by stock, and evidently discarded, for it was lying about on the surface in small, dead tufts. *Agrostis* was closely eaten-down on the treated portions of the field, where it was growing in association with *Cynosurus*, *Festuca*, and *Trifolium repens*. On the untreated area it was growing in coarse tufts, and was ungrazed and altogether different in appearance from that on the improved land. On the latter, *Agrostis* was undoubtedly preferred by stock to *Holcus* and *Anthoxanthum*—in fact it was eaten as readily as any other grass growing on the treated area. *Triodia* was very little touched by stock.

Case B. A rough pasture, an "in-take" from the neighbouring heathered moor, 80 acres in extent. Altitude, about 600 ft. rising to 800 ft. Soil, variable, but on the lower half, including the improved portion, a rather light loam formed from a gritty drift of Boulder Clay overlying Milltone Grit. Southern aspect. The lower half is fairly dry, the upper is more level, and wet, and covered with several inches of black peat. Since the upper portion differs markedly in both physical

character and botanical composition from typical White Bent land, the following observations are confined to the lower half only. The whole field was valued in 1902 at 5*s*. per acre rent.

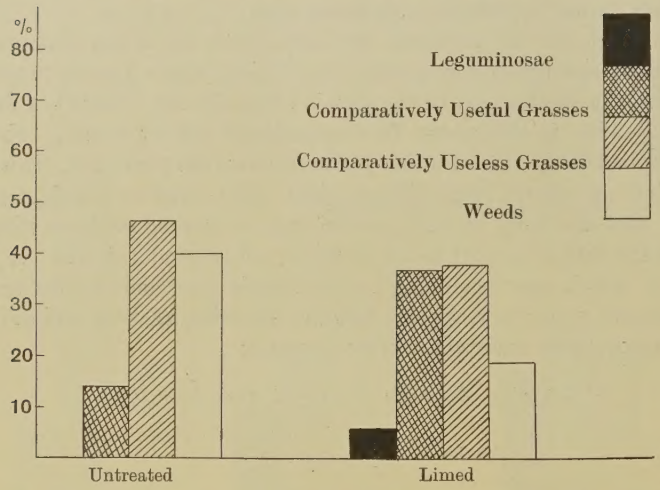
Although on the untreated dry area there are a few damp spots formed by springs emerging from the hillside, where *Juncus communis* appears, the greater part of the area is naturally dry. There is a mat of from 1½–2 ins. in thickness. To a rectangular plot of 6 acres, cob-lime was applied at the rate of 6 tons per acre about the year 1862. This area standing out clearly from hilltops eight miles away as a bright green patch, and also being closely grazed, forms a marked contrast with the rest of the field. The herbage is, however, not so good as it was ten years ago. A mat is now beginning to form again and some of the coarser grasses and weeds to reappear; but the following analysis will indicate the improvement maintained after 60 years:

Botanical Analysis of Case B, 18th July 1922.

Species	Untreated %	Limed 1862 %
Leguminosae:		
Trifolium repens ...	—	5·7
Comparatively useful grasses:		
Festuca ovina ...	14·0	36·1
Dactylis glomerata ...	—	·4
*Achillea millefolium ...	—	·3
Comparatively useless grasses:		
Nardus stricta ...	32·0	—
Agrostis sp.	9·5	26·8
Holcus lanatus ...	1·0	5·7
Anthoxanthum odoratum	·4	1·2
Molinia caerulea ...	1·0	—
Triodia decumbens ...	·3	—
Poa compressa ...	2·0	4·2
Weeds and bare space:		
Galium saxatile ...	13·0	9·1
Potentilla tormentilla ...	3·3	—
Euphrasia officinalis ...	Trace	Trace
Rumex acetosa ...	·2	·7
Cnicus arvensis ...	—	·5
Polygala vulgaris ...	Trace	—
Aira caespitosa ...	9·0	·2
Juncus communis ...	7·0	—
Luzula campestris ...	3·9	6·8
Carex sp.	·1	Trace
Bare space	3·3	1·4
Leguminosae	Nil	5·7
Comparatively useful grasses	14·0	36·8
Comparatively useless grasses	46·2	37·9
Weeds and bare space ...	39·8	18·7
Totals ...	100·0	99·1

* See footnote to first table.

Diagram to illustrate analysis of Case B.



The difference in the quality of the *Agrostis* growing on the untreated and on the limed areas was very noticeable; that on the former being ungrazed, brown and tough; that on the latter being closely eaten down, green, sweet smelling, and evidently much favoured by stock. The clover appeared to be losing vigour, and was undoubtedly present in greater quantity 10 or 15 years ago.

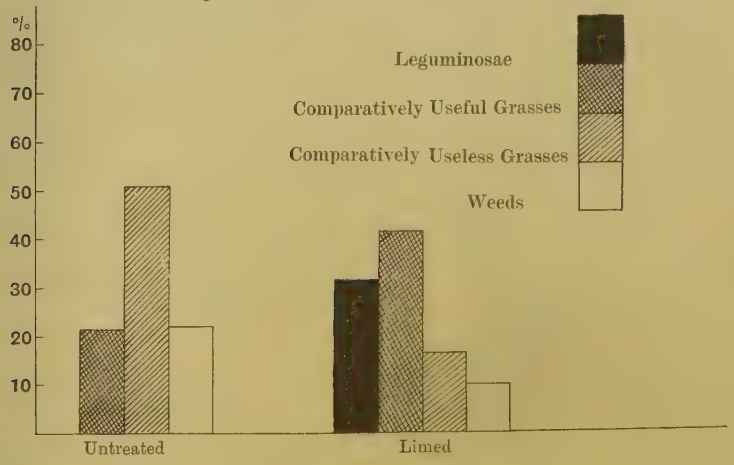
Case C. A rough pasture 24 acres in area. Altitude, 600 ft. Situated on a southern slope. Soil, a gritty loam, overlying Millstone Grit, and dry. A portion of the field, 5 acres in area, was limed in 1911, at the rate of 5 tons per acre small lime. At a distance this plot could be clearly distinguished from the untreated part of the field by its fresh green colour. On the untreated area the grass was 8–10 ins. long, brown and stemmy, interspersed with patches of rushes; and there was a mat 2–3 ins. in thickness. The limed plot on the other hand was, as usual, closely grazed, the rushes had disappeared, as also had the mat. The rental value of the unimproved land was estimated by the tenant at 5*s.* per acre, that of the improved at 25*s.* Another part of the field was limed in March 1921, and in August 1922 a difference was already apparent in the herbage, and clover was appearing. It was noticeable that stock already preferred this newly-limed area to the untreated.

Botanical Analysis of Case C, 14th August 1922.

Species	Untreated %	Limed 1913 %
Leguminosae:		
Trifolium repens	—	23·0
Lotus corniculatus ...	—	8·3
Comparatively useful grasses:		
Festuca ovina et duriuscula	20·8	32·0
Dactylis glomerata ...	—	1·1
Phleum pratense ...	—	·9
Poa pratensis	—	1·3
Cynosurus cristatus ...	·5	4·1
Trisetum flavescens ...	—	1·1
*Plantago lanceolata ...	—	1·0
Comparatively useless grasses:		
Nardus stricta	37·2	·8
Agrostis spp.	7·1	11·0
Holcus lanatus	4·3	2·6
Anthoxanthum odoratum	·6	·6
Triodia decumbens ...	1·8	1·2
Weeds:		
Galium saxatile	·7	—
Potentilla tormentilla ...	12·8	·5
Euphrasis officinalis ...	—	·5
Rumex aceotsa	·9	—
Ranunculus sp.	—	·4
Hieracium pilosella ...	—	·7
Prunella vulgaris ...	Trace	·2
Viola sp.	—	1·2
Cerastium vulgatum ...	—	·4
Aira caespitosa	1·9	·8
Juncus communis ...	2·5	—
Luzula campestris ...	6·0	2·4
Carex sp.	2·1	2·2
Moss	·1	·5
Leguminosae	Nil	31·3
Comparatively useful grasses	21·3	41·5
Comparatively useless grasses	51·0	16·2
Weeds	27·0	9·8
Totals ...	99·3	98·8

* See footnote to first table.

Diagram to illustrate analysis of Case C.



Throughout the district a number of other cases of the treatment of White Bent land have been observed, and the results recorded are in entire agreement with those cited in the foregoing examples. Owing to lack of space, the briefest details only can be furnished of three further typical instances, as under:

Case D. A rough pasture covered with a 1½–2 in. mat, growing mainly *Nardus*, *Galium* or *Potentilla*, with an admixture of *Festuca ovina et duriuscula* but no *Trifolium repens*. Soil, medium loam, overlying Mountain Limestone; fairly dry. Six tons per acre of cob-lime applied in 1912 to a portion of the field has removed the mat, eradicated the *Nardus*, induced the growth of clover, and increased the rental value from 5*s*. to 25*s*. per acre.

Case E. A high-lying, exceedingly rough pasture. Soil, heavy loam overlying Millstone Grit. Three and a half tons of small lime per acre applied to part of the field in 1912 has converted a pasture worth about 1*s*. per acre into one worth 20*s*. It is interesting to note that one of the chief constituents of the improved herbage is *Agrostis*.

Case F. A rough pasture. Soil, medium loam, overlying Millstone Grit, covered with a 1½–2 in. mat, and growing mainly *Nardus*, *Festuca ovina*, *Galium* and *Luzula*. High-grade slag applied at the rate of 10 cwts. per acre to part of the field in 1915 has produced no evident result. Gas-lime applied to another portion of the field, has largely eradicated the *Nardus* and *Galium* (which have been replaced mainly by *Festuca ovina*), and has produced a sweet, green pasture.

It will be noticed that, with the exception of Case E, the dressing of lime in the above examples was 5 tons per acre or more. In such cases a layer of lime is found from 3–6 ins. below the surface of the soil. From this fact and the study of Case E, together with general observations, there appears to be good reason to believe that, even on the roughest pastures, so moderate a dressing as 3 tons per acre of good quality cob-lime is sufficient to break up the mat, to effect a valuable improvement in the herbage, and to prepare the way for an excellent result from slag. It is possible that this point will be demonstrated by experiments which were laid down by one of the authors in the Skipton district in 1923, under the auspices of Leeds University.

The foregoing studies appear to justify the following conclusions in regard to the treatment of White Bent land:

(1) A big and lasting improvement can be effected on such land, if reasonably dry, by suitable applications of lime.

(2) Slag applied after lime will still further improve such land.

(3) Slag without lime will effect no improvement.

(2) *Agrostis pasture.* Pastures in which the chief constituent of the herbage is *Agrostis* occupy considerable areas in the West Riding. They are found overlying Mountain Limestone, Millstone Grit, River Alluvium and Boulder Clay formations. With *Agrostis* are usually associated *Festuca ovina*, *Galium*, *Potentilla* and *Luzula*. Such pastures are usually matted, but, in the districts under consideration, *Agrostis* mats are not generally so thick as those associated with *Nardus*. It is to be noted also that *Agrostis* mats are as a rule more open in texture, and often have a considerable amount of soil admixed with them; they are consequently not so impenetrable to rain and to the roots of plants as are mats of White Bent.

The kind of treatment to which *Agrostis* pasture responds depends largely on the thickness and texture of the mat, together with the lime requirement. In some cases basic slag alone will effect a satisfactory improvement, whereas in others a previous dressing of lime is essential. Dr Hanley (Leeds University) informs us that, in his experience, basic slag is generally effective on such pastures where the lime-requirement is less than 30 cwts. per acre; that its action is doubtful where the lime-requirement lies between 30 and 40 cwts.; that it is not effective when the lime-requirement exceeds 40 cwts.; and that from an inspection of the mat and a knowledge of the lime-requirement, it is often possible to determine whether slag will be effective or not. The following two cases are illustrative of these remarks:

Case G. An old pasture covered with a $1\frac{1}{2}$ in. mat, and with a lime-requirement of 30 cwts. per acre. Soil, a heavy loam inclined to be damp, derived from Millstone Grit Shale. Altitude, 850 ft.

In February 1920 three plots, each half an acre in area, were laid down by the University of Leeds, as follows:

Plot 1. Lime 2 tons per acre.

Plot 2. Basic slag, 38 per cent., 10 cwts. per acre.

Plot 3. Untreated.

In April 1920 a cross dressing of 4 cwts. per acre of Kainit was applied over one-third of the plots; and in November 1921 a cross dressing of basic slag, at the previous rate, was applied over another one-third[1].

Frequent visits have been made to these plots since January 1921. It was noticed that the basic slag plot was the first to show differentiation, the percentage of clover in it visibly increasing during the summer of 1921, and still more so in 1922; and it was more closely grazed than the other plots. In August 1922, when the analysis was being made, the

[1] See *Guide to Grassland Demonstrations in Yorkshire.*

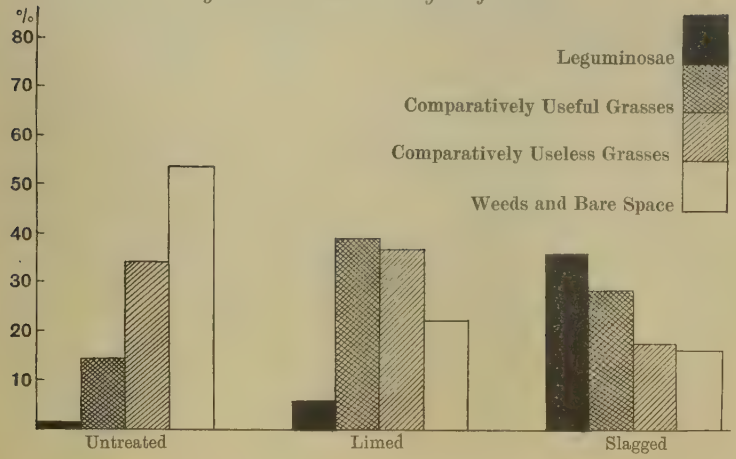
contrast between this plot and the others was very marked; it was eaten bare by stock, the greater part of the mat had disappeared, and the clover looked particularly healthy and vigorous and was spreading rapidly. The limed plot, on the other hand, had not been so well grazed, was still considerably matted, and contained but little clover. It is probable that the lime had not yet had sufficient time to produce anything like its maximum effect. *Agrostis* on the limed and untreated areas was not much grazed by stock, although on the slagged plot it had been readily eaten down.

Botanical Analysis of Case G, 19th August 1922.

Species	Untreated %	Limed 1920 %	Slagged 1920 %
Leguminosae:			
Trifolium repens ...	·9	4·8	33·5
Trifolium pratense ...	—	Trace	—
Lotus corniculatus ...	·5	·8	2·5
Comparatively useful grasses:			
Lolium perenne ...	1·1	—	·6
Festuca ovina ...	9·0	28·6	15·2
Dactylis glomerata ...	—	5·0	—
Phleum pratense ...	—	2·0	2·0
Cynosurus cristatus ...	2·2	3·1	7·2
*Plantago lanceolata ...	2·0	·5	3·6
Comparatively useless grasses:			
Nardus stricta ...	—	1·1	·5
Agrostis spp.	26·1	27·3	16·0
Holcus lanatus ...	2·5	1·4	—
Anthoxanthum odoratum	·7	·9	·1
Triodia decumbens ...	·9	—	1·1
Briza media	·2	—	—
Weeds and bare space:			
Galium saxatile ...	—	·4	—
Potentilla tormentilla ...	2·7	—	—
Rumex acetosa ...	·7	1·0	—
Ranunculus acris ...	1·8	1·2	·3
Hieracium pilosella ...	—	·9	·9
Leontodon sp. ...	5·6	3·0	2·0
Bellis perennis ...	1·1	—	1·0
Centaurea nigra ...	·7	—	—
Prunella vulgaris ...	—	—	·5
Campanula rotundifolia	2·7	—	—
Aira caespitosa ...	4·7	4·0	·7
Luzula campestris ...	32·2	3·3	3·5
Carex sp.	1·3	·8	—
Moss	—	3·0	2·2
Bare space	—	4·9	5·0
Leguminosae	1·4	5·6	36·0
Comparatively useful grasses	14·3	39·2	28·6
Comparatively useless grasses	30·4	30·7	17·7
Weeds and bare space ...	53·5	22·5	16·1
Totals ...	99·6	98·0	98·4

* See footnote to first table.

Diagram to illustrate analysis of Case G.



Analyses were made of the plot which received only the initial dressing of slag in 1920, and of the lime-alone plot; and the preceding table indicates the differences in herbage induced by these dressings. An analysis of the untreated area made by Miss L. I. Scott, B.Sc., Leeds University, in 1921, is given above; and, although it is realised that analyses made in different seasons are not strictly comparable, it is felt that these figures have some value as indicating the original nature of the herbage.

Case H. A rough hill pasture 21 acres in area, covered by a 2 in. mat and sloping steeply to the south. Mean altitude, 850 ft. Soil a good loam, varying in depth from 1–2 ft., immediately overlying limestone, and dry. In 1908 small lime was applied to 7 acres of this field, in parallel strips running across the slope, at the rate of $6\frac{3}{4}$ tons per acre. The cost of the lime was then 3*s.* 9*d.* per ton.

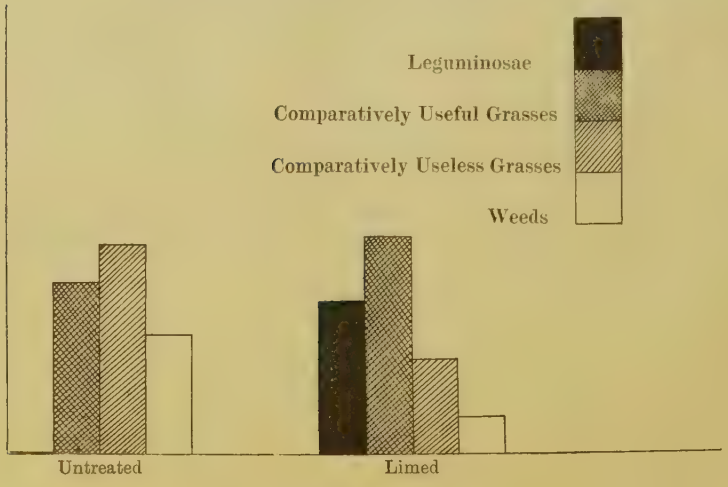
In August 1922 the untreated area was red with the flowering heads of *Agrostis*, whereas on the limed strips few flower stalks could be found, and the herbage was sweet and closely grazed. As in other cases, the *Agrostis* persisting on the limed portions seemed to possess an altogether superior economic value to that on the untreated land. The tenant values the limed area at 22*s.* 6*d.* per acre rent and the unlimed at 7*s.* 6*d.*

Botanical Analysis of Case H.

Species	Untreated %	Limed 1908 %
Leguminosae:		
Trifolium repens ...	—	16·8
Lotus corniculatus ...	·1	12·9
Comparatively useful grasses:		
Lolium perenne ...	—	1·8
Festuca ovina et duriuscula	32·0	35·9
Cynosurus cristatus ...	—	1·0
*Achillea millefolium ...	1·4	3·8
Comparatively useless grasses:		
Agrostis spp.	34·8	10·7
Holcus lanatus... ...	·4	4·3
Anthoxanthum odoratum	2·8	3·0
Triodia decumbens ...	3·1	·8
Weeds:		
Galium saxatile ...	12·9	—
Galium pusillum ...	—	1·8
Potentilla tormentilla ...	5·5	·1
Rumex acetosa ...	·4	—
Ranunculus sp. ...	—	·4
Cnicus arvensis ...	—	Trace
Prunella vulgaris ...	—	·1
Campanula rotundifolia ...	—	·2
Euphrasia officinalis ...	—	Trace
Viola sp.	·2	1·4
Thymus serpyllum ...	—	·1
Aira caespitosa ...	—	·3
Luzula campestris ...	4·1	1·7
Carex sp.	·3	·8
Moss	—	·2
Leguminosae	·1	29·7
Comparatively useful grasses	33·4	42·5
Comparatively useless grasses	41·1	18·8
Weeds	23·4	7·1
Totals ...	98·0	98·1

* See footnote to first table.

Diagram to illustrate analysis of Case H.



Brief notes of other examples of the treatment of *Agrostis* pasture are herewith presented:

Case J. A field with a 1–1½ in. mat, on medium grit, growing mainly *Agrostis* with *Galium*, *Potentilla*, *Festuca ovina* and a little *Cynosurus*. Cob-lime at the rate of 5 tons per acre applied to a part of the field in 1919 has eradicated the mat, consideraby decreased the *Agrostis*, and induced a healthy growth of clover. Another area received 12 cwts. per acre of 35 per cent. basic slag, which has produced a similar but less marked improvement.

Case K. A poor pasture with a 1½ in. mat. Soil, a good loam, 12–18 ins. deep, overlying Mountain Limestone. Herbage mainly *Agrostis* intermixed with *Festuca ovina* and *Galium*. High-grade slag applied at the rate of 10 cwts. per acre in 1913 has almost eradicated the mat, induced the growth of *Trifolium repens*, *Cynosurus*, and a small quantity of *Lolium perenne*. A large quantity of *Agrostis* still remains, but the grass now most in evidence is *Festuca ovina*. This field gives one the impression that, although slag has effected a big improvement, it has been hindered in its action by the deficiency in lime and the presence of the mat; and that a better result would have been obtained had there been a previous light dressing of lime.

Case L. A poor pasture with a 1–1½ in. mat, growing chiefly *Agrostis*, *Festuca ovina*, *Galium*, *Potentilla* and *Luzula*. Soil derived from Millstone Grit. Applied to certain areas in 1917 and to others in 1919, small lime at the rate of 25 cwts. per acre has produced a marked improvement. Even this light dressing has eradicated the mat, and although clover is not prominent, small starved plants are present everywhere. In December 1922 there was every indication that a dressing of slag would produce excellent results.

The evidence presented by the foregoing studies is somewhat conflicting, and shows that the treatment of *Agrostis* pasture is not so straightforward as that of White Bent land. While it is realised that further work is required before any definite conclusions can be drawn, our observations tend to confirm Dr Hanley's opinions as quoted on p. 145. Where the mat does not exceed 1½ ins. in thickness basic slag will effect an improvement, which may be either inferior (see Case J) or superior (see Case G) to that produced by lime alone. Where the mat is as thick as 2 ins., even on soil overlying limestone, lime will produce a satisfactory result.

II. BETTER-CLASS PASTURE.

Under this heading may be included those lower-lying and productive tracts of land which are found in the valleys and on the lower slopes of the hills of the West Riding. Such fields occur commonly on River Alluvium, on the Mountain Limestone formation where there is a sufficient depth of good soil, on Boulder Clay, and, less frequently, on Millstone Grit. Briefly speaking, applications of basic slag seldom fail to effect improvement on the heavier and damper soils; whereas on drier soils, inclined to be sandy, steamed bone flour is usually more successful. The fact that on medium and lighter land basic slag is not always efficacious may, in many cases, be attributed to the deficiency in lime occasioned by the heavy rainfall of the district. Lack of lime in such fields is indicated by the presence of a mat, which in good pastures may vary from a trace only observable here and there on the rougher portions of the ground, to 2 ins. in thickness on poorer fields. As a rule, where the mat exceeds $1\frac{1}{2}$ ins. in depth, an initial light dressing of lime is required, which may be followed by the usual application of slag. Slag alone, without lime, sometimes fails to evoke any response whatever. A striking example of the efficacy of lime in improving a pasture which, although not of the best, was still far from being poor, is herewith described.

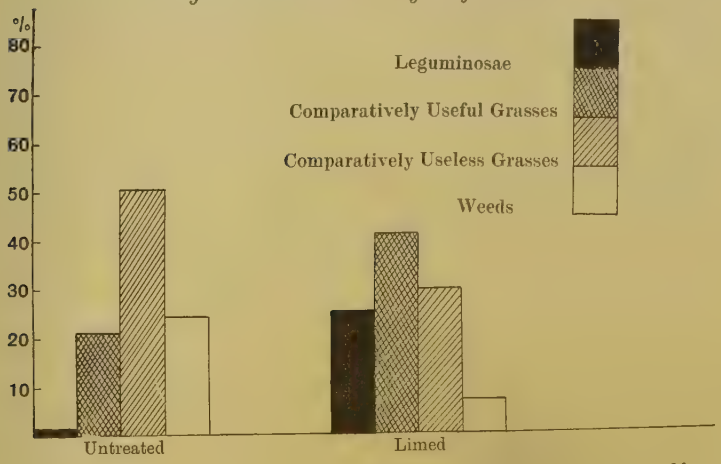
Case M. A field of 70 acres, on a south-eastern slope. Altitude, 400 ft. Soil, a medium loam, derived from a gritty drift of Boulder Clay. Considerable variations occur in the field, the eastern area having poorer herbage and a 1–$1\frac{1}{2}$ in. mat. The botanical analysis which follows applies to this portion only, two acres of which was limed in 1914, at the rate of 5 tons per acre cob-lime. This application has effected a most conspicuous improvement, the herbage on the limed area starting into growth much earlier and being green and closely-grazed throughout the season. The lime-requirement of the improved area is 20 cwts. and that of the untreated 42 cwts. Other strips of this poorer part of the field have been treated with basic slag with no result. When the field was analysed it was noticed that, as in other cases, the *Agrostis* on the limed area was as closely grazed as the better grasses, green and sweet-smelling; whereas that on the untreated area was ungrazed, reddish-brown, tough and wiry.

Botanical Analysis of Case M, 15th July 1922.

Species	Untreated %	Limed 1914 %
Leguminosae:		
Trifolium repens ...	·9	25·2
Lotus corniculatus ...	·6	—
Comparatively useful grasses:		
Lolium perenne ...	·8	2·7
Festuca ovina ...	14·1	19·3
Dactylis glomerata ...	5·3	3·3
Poa trivialis	·5	9·9
Trisetum flavescens ...	·2	3·0
Cynosurus cristatus ...	—	3·0
*Achillea millefolium ...	·3	—
Comparatively useless grasses:		
Agrostis spp. ...	41·9	17·5
Holcus lanatus ...	5·5	7·0
Anthoxanthum odoratum	3·1	1·4
Weeds and bare space:		
Potentilla tormentilla ...	1·5	—
Rumex acetosa ...	6·0	1·2
Ranunculus sp. ...	·6	1·0
Cnicus arvensis ...	1·1	3·4
Aira caespitosa ...	·2	—
Luzula campestris ...	7·6	1·3
Bare space	7·4	—
Leguminosae	1·5	25·2
Comparatively useful grasses	21·2	41·2
Comparatively useless grasses	50·5	25·9
Weeds and bare space ...	24·4	6·9
Totals ...	97·6	99·2

* See footnote to first table.

Diagram to illustrate analysis of Case M.



The above case illustrates the fact that even better-class pastures in the district under consideration respond in a remarkable way to applications of lime, the increase in the growth of clover being particularly striking. It should be remarked that Case M is by no means an isolated instance of such improvement.

Our thanks are due to Dr J. A. Hanley for his kindness in taking soil-samples and furnishing lime-requirements, and for other valuable assistance; and to L. A. Thompson, M.Sc., for giving us details of his own researches into pasture-improvement in Northumberland.

SUMMARY.

There are large areas of rough hill pasture in the West Riding of Yorkshire which are covered with a fibrous mat of undecomposed vegetable matter 1–3 ins. thick, and whose herbage falls naturally into two types, according to whether *Nardus* or *Agrostis* predominates. On such land lime forms an important ameliorative agent, breaking up the mat, decreasing the *Nardus*, encouraging the finer grasses, and inducing a vigorous growth of clover.

It is shown that on reasonably dry land growing from 30–40 per cent. of *Nardus stricta*, 15–20 per cent. of *Festuca ovina*, and a conspicuous amount of *Galium saxatile*, *Luzula campestris* and *Potentilla tormentilla*, suitable applications of lime effect a striking improvement, which may be maintained for as long as 60 years; that lime followed by basic slag produces still better results; that basic slag alone has no effect.

Where *Agrostis* predominates the mat is more open in texture and usually thinner than that associated with *Nardus*. On such land a satisfactory improvement is effected in certain cases by lime; and in others, where the mat does not exceed $1\frac{1}{2}$ ins. in thickness, by basic slag alone. A great change is observed in the quality and palatability of the *Agrostis* as a result of such treatment.

Even on lower-lying and better-class pastures in this district most beneficial results may be obtained from dressings of lime.

(*Received March 7th*, 1924.)

THE DENSITY OF UNICELLULAR ORGANISMS

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(With 6 Text-figures.)

In biological studies of the soil or other materials, where it is desirable to calculate the total mass of a population from quantitative data representing numbers and dimensions, an accurate knowledge of the density of the particular cell or cells concerned is of considerable importance. The literature involving studies of this type seems to indicate that the values that have been applied to this purpose are largely speculative since, in no instance noted, have they been supported by definite experimental data.

The present investigation has grown out of studies upon the capacity of the soil for the adsorption of certain unicellular organisms. In this work some of the cells mentioned in the present paper were used. The relation of the two developed as a result of the technique applied in the adsorption studies. This has been essentially as suggested by Cutler(2) earlier, and consists in shaking a definite quantity of soil with varying numbers of the cells under study and, after the suspension has settled for 3–4 minutes, in order to permit the coarser particles and floccules to fall out, counting directly the number of cells remaining in free suspension with the aid of an haemocytometer. Since in these studies only a gram or less of soil is used with but 1–2 c.c. of the suspended organisms, a knowledge of the rate of fall of the individual cells would be very useful in determining just how the sample of the supernatant liquid should be withdrawn to represent the true concentration of the cells remaining in suspension. This part of the procedure will be treated in greater detail at a later time.

In the present work, both plant and animal cells have been studied. For the former, a pure culture of unicellular alga was kindly supplied by Dr Bristol of the Mycology Department. This particular species (*Scenedesmus*, number D) was used by Bristol and Page(1) in their recent studies upon nitrogen fixation. Yeast (*S. cerevesiae* and *glutinus*) and

spores of *B. mycoides* were also studied in this way. Since the latter studies are still incomplete, only the results with the algal cells will be considered in any detail at the present time. For the animal cells, the cysts of protozoa alone were studied. The cysts of *Hartmanella hyalina*, *Colpoda* sp. and *Gonostomum* sp., were used for this purpose. The first was supplied in pure culture by Mr Cutler, and the second by Mr Sandon, while the third was obtained by the writer from a sample of Utah soil supplied through the kindness of Prof. C. T. Hirst of the Utah Agricultural Experimental Station. The stock culture of alga was grown in the standard solution suggested by Bristol and Page(1) which included together with small quantities of other salts, calcium nitrate as a source of nitrogen and glucose as a source of energy. When grown upon a solid substratum, the same basic medium was used with the addition of 1·5 per cent. agar-agar. The protozoa were grown in petri dishes upon a nutrient agar substratum upon the surface of which 1–3 c.c. of water was maintained.

PROCEDURE.

It was found that the first necessity for a study of this nature was an observation cell in which, after filling and mounting upon the microscope, the suspending medium should be, as nearly as possible, devoid of all superficial movements or currents. This condition was finally obtained by the use of two heavy glass slides 4 mm. in thickness between which, on either side, two strips of glass 7·5 mm. broad and 1·2 mm. thick were sealed. This then gave a cell 9 cm. long, 1 cm. broad and 1·2 mm. in depth. To the open ends of the cell so formed, an outlet and inlet constructed of heavy capillary tubing, were sealed. Both of these parts were made as short as possible and the former drawn out to a small opening in order to reduce the exposure of the liquid to a minimum. It was found best to keep both the inlet and outlet tubes horizontal with the top of the cell. After filling, care being taken to remove all air bubbles, a solid glass tube was inserted into the opening of the inlet—a short piece of rubber tubing being previously mounted upon this part. Thus there was left exposed but the small opening at the outlet which could, when desirable, be easily protected by placing a cap over it.

After insulating the observation chamber against direct contact with metal surfaces, it was mounted upon the movable stage of a microscope which, for this purpose, was always placed in a horizontal position. Thus, there was available for observation and measurement, a distance of 1 cm. fall, with a focal depth of 1·2 mm.

In order to avoid the effect of heating from the light used, illumination was effected by the use of a 40-watt bulb suspended in a sealed, cylindrical glass jar which in turn was placed in a large square battery jar and water from the tap circulated about the whole inner compartment thus constituted. The outer jar was covered with heavy paper with the exception of a round, ground glass window immediately before the microscope. This latter permitted focusing through the cell directly into the light without the use of a condenser. With walls of such thickness, it was not possible to use a high power objective, but by using a 16 mm. Spencer lens objective and drawing the tube of the microscope to its maximum length, a magnification of about 400 diameters was obtained and proved entirely satisfactory for the measurement both of the velocity and dimensions of all cells studied.

In random measurements of individual cells for both velocity and dimensions, it was found necessary, particularly in the case of the smaller ones, to devise a method of measuring their diameter as they fell through the suspending medium towards the bottom of the observation chamber. The larger protozoan cysts, which fell rather too rapidly for accurate measurement in the manner to be described, were measured as they rested upon the bottom.

For the purpose of measuring the diameter of the smaller cells under the conditions indicated, a Spencer micrometer eye-piece (number 425), with vernier and movable scale was used. It was found possible to mount immediately about this movable scale, a simple micrometer eye-piece scale which, by virtue of its position would be stationary in relation to the movable scale. Due to its proximity to the latter, both scales were in clear focus at all times. Both the movement of the vernier and the fixed scale were calibrated against a standard stage micrometer. Thus by the use of the movable stage of the microscope, one side of the falling cell could be held to a certain position against the fixed scale in the eye-piece while the movable scale was adjusted to the other side. With the diameter registered upon the two scales as the distance between a point upon each respectively, by the manipulation of the vernier, the actual diameter in mm./1000 could be subsequently read off at leisure.

The velocity of the particle was usually ascertained prior to the measurement of its diameter. This was accomplished, with the aid of a stop-watch, as the cell fell vertically past the fixed scale. It was readily possible, however, by placing the two scales at right angles to each other to take both measurements simultaneously. Since in these measurements the tube of the microscope was fully extended and could not easily be

held entirely rigid, the very slight movement which almost unavoidably accompanied the manipulation of the vernier, made the former procedure of making the measurements independently the more desirable. It might be noted that if the measurements were made simultaneously, since the whole micrometer with both scales could be rotated in the barrel of the microscope, it was possible to measure either of the apparent dimensions of an object, moving under these conditions, regardless of the direction, or within certain limits, the rate of its movement.

In the actual determination of the velocity of fall, it was found that great care was required in ascertaining the equilibrium, both in the suspending medium and in the rate of fall itself. For while two consecutive measurements of the rate for the same cell through 60 or 80 seconds may give results sufficiently close in their agreement to be accepted as

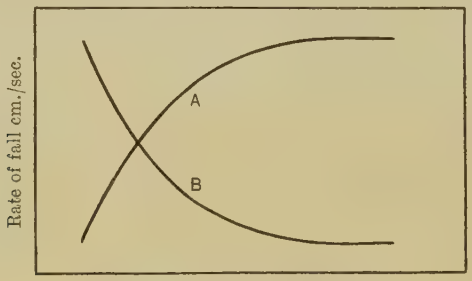

Fig. 1. Curves showing general tendency of velocity changes previous to the appearance of fluid equilibrium.

reliable duplicates, not infrequently, in a longer series of successive measurements it has been found that such a disagreement, though slight, continued in a definite direction. The nature of this tendency will be made clear by reference to Fig. 1. Thus, if a series of successive measurements be plotted as time against the rates observed at each interval, it may require a considerable period before the rate of fall actually approaches a constant value and the fluctuations become truly random in nature rather than unidirectional. The direction of the change in the rate of fall is by no means always the same. Sometimes it increases to a constant rate as shown at *A* and at other times it diminishes as indicated at *B* (Fig. 1). The nature of these effects depends, seemingly, upon the extent and direction of the currents naturally imparted to the fluid mass at the time of filling the cell.

Since a rather marked retardation to the free movement of the cells

has been observed near the boundary of the fluid and glass, all measurements have been made within the middle quarter of the observation chamber. The determinations recorded below are based upon duplicate or triplicate measurements of the diameter and from four to six or eight measurements of the velocity after it had become constant. When the mean square error was calculated upon the basis of forty-three duplicate measurements of the velocity and diameter of algal cells taken at random, it was found that for the former it was $\pm$ 0·0445 and for the latter $\pm$ 0·01588.

CONDITIONS OF EXPERIMENT.

In order to control the conditions under which the observations were made from day to day or hour to hour, as closely as possible, all measurements were made in the constant temperature room of the Physics Department in which the automatic self-recording balance is kept. In this way, viscosity changes due to temperature could be readily controlled to one-tenth of a degree. The constancy of the temperature is also an important factor in the elimination of convection currents due to temperature changes in the boundary layer of the liquid medium which is in contact with the glass walls of the observation chamber.

Since the cells could not be studied in distilled water, tap water was finally adopted as the suspending medium and was used in all cases. Injury to any of the cells was not observed at any time through its use. The water was drawn in quantity and allowed to come to room temperature before using. Its density was also carefully determined for a series of temperatures on either side of the average temperature at which it was to be used. In this, the tables of P. Chappius (*Bureau internat. des Poids et Mesures, Travaux et Memoires* XIII, 1907) were used as reference. It was found that the density of the tap water at the average temperature of the experiments (12·4° C.) was much nearer unity than that of distilled water under the same conditions. At 12·4° C., the actual density of the tap water used was found to be 0·99989 or equivalent to that of distilled water at 7·7° C. The density of distilled water for the indicated temperature is 0·99948. Since the records of actual cell density are given but to the third decimal place, the slight correction required through the use of tap water as a suspending medium has not been necessary.

EXPERIMENTAL RESULTS.

The formula of Stokes in which

$$V = \frac{2}{9}\frac{r^2(\rho - \rho_1)g}{\mu},$$

where V = velocity,
r = radius,
ρ = density of particle,
ρ_1 = density of suspended liquid,
μ = coefficient of viscosity of suspending medium,
g = gravity constant,

provides that with a knowledge of the rate of fall of a body of known radius in a liquid of known density and viscosity, then the density of the particle, within certain limits of size for particles of given density, can be calculated therefrom, since $\rho_1 - \rho = \frac{9u - V}{2r^2 g}$. All calculations are made with measurements in terms of C.G.S. units. For these studies the coefficient of viscosity of the medium was taken as for distilled water and the value for the temperature desired was obtained by interpolation by means of the formula of Everett(3) into the table of these values given by Kaye and Laby(4).

As an instance of the nature of the actual measurements performed, the average data obtained for large and small algal cells will be contrasted with similar measurements upon *Gonostomum* cysts. In view of the nature of the results given in more detail below, it would seem that the application of Stokes' formula to these studies is fully justified.

	Radius (cm.)^2	Rate of fall (cm./sec.)	Density $1+(\rho-\rho_1)$
1. Alga	·000000052	·000134	1·146
2. „	·000000693	·000802	1·071
3. *Gonostomum* cysts	·00000218	·00252	1·065
4. „ „	·00000580	·00561	1·054

From theoretical considerations Mr Fisher of the Statistical Department has found that for particles of the nature and density of those used in these experiments, the maximum particle diameter for which the formula might be expected to hold would be in the neighbourhood of 200μ. The largest particles used in these studies, however, were less than 50μ, thus leaving a wide margin of safety. With regard to the position, in this respect, of the particles of smaller size, it might

be noted that for definite physical reasons the applicability of the formula becomes progressively less strained as the diameter of the particles decreases.

THE DENSITY OF UNICELLULAR GREEN ALGAE.

The distribution of about 120 random measurements are shown in Fig. 2 where the continuous black line represents the main axis passing through the vertex. The radius of the individual cell is plotted against the square root of its velocity, and it is seen that the position of any particles of given size, though varying widely in velocity, follows the main axis in a thoroughly consistent manner. The mean values calculated for the cells of different sizes, as grouped in Fig. 3, have been plotted as the broken line. In Fig. 3 the correlation between the mean density of the cells, as calculated from the mean radius and velocity, and their radius (curve *A*) is shown, together with the distribution curve for the sizes of the individuals. There is a consistent variation in density with size of the cells, that of the smaller being considerably greater than the medium or larger individuals.

The mean density is found to range from 1·07 in the case of the larger cells to nearly 1·15 in the case of the smaller.

The flat portion of the density curve is seen to correspond with that portion of the mean curve in Fig. 2 which most closely follows the axis. By analysing the relationship between the density and distribution curves it is seen that the larger part of the organisms studied are distributed through the flat portion of the former curve. Through this entire section from $r = 4$ to $r = 7$, the density only varies 0·006, and this portion of the curve represents 85 per cent. of the cells measured. Taking into account the distribution of the measurements with regard to size, the mean density is found to be 1·098, which is in good agreement with the value 1·090 found to fall upon the median of the distribution curve.

Without doubt the observed decrease in density, with increase in size of the algal cells, may be attributed to fundamental changes in the physical and chemical nature of the protoplasm as these organisms approach maturity. Thus, either the development of vacuolar structures in the protoplasm or the formation of oils, both of which are definitely known to occur, would serve to lighten the cell, as a whole, very materially. Under certain conditions where the cell contents are in an advanced stage of division and the cell wall is slightly distended with corresponding spaces within the cell wall unoccupied by protoplasm, the apparent density might be considerably decreased. In the present studies, however,

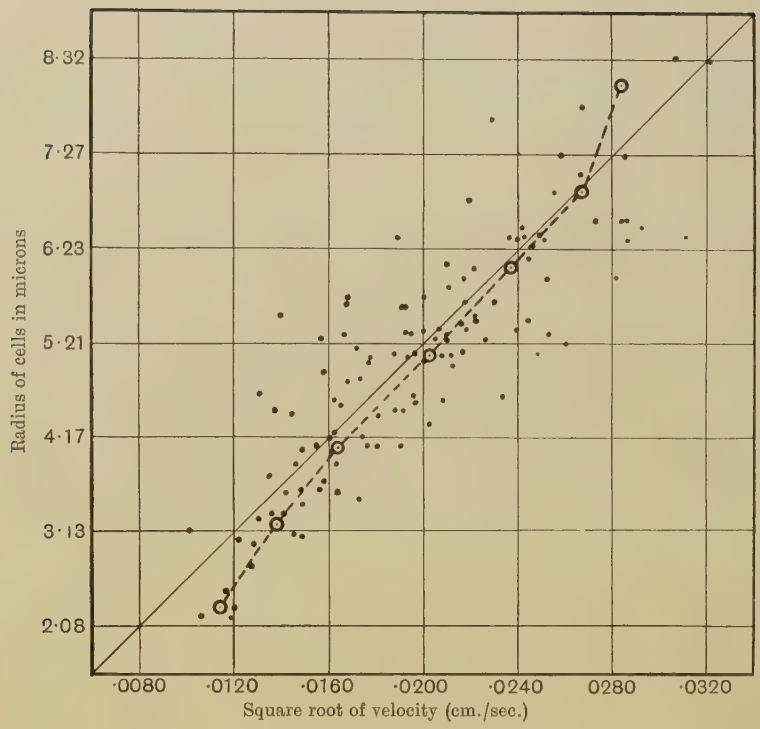

Fig. 2. A dot diagram with regression curve of average values for velocity and diameter.

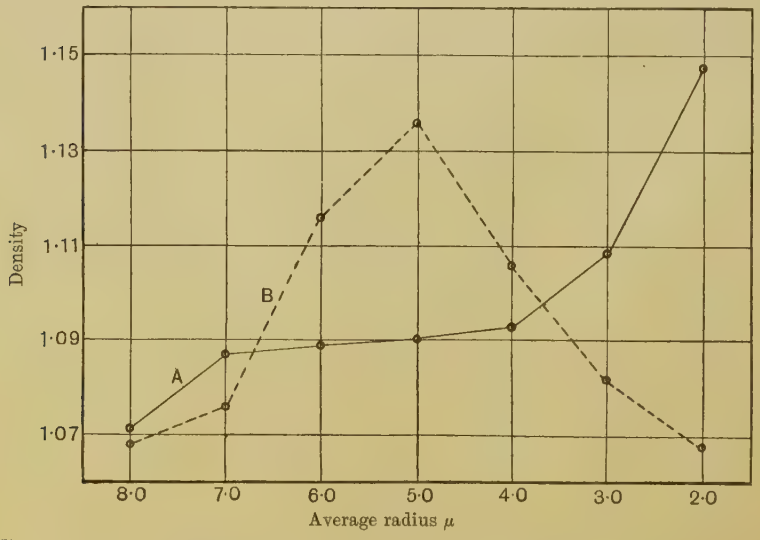

Fig. 3. The correlation (curve *A*) of density of algal cells with size, showing at (*B*) the distribution curve of the individuals measured.

measurements were not made upon cells where division was apparent in any way.

The fact that the density of the cells seemed to decrease so materially with age and development led to further investigation. Measurements were made upon a series of individuals from a culture which was three months old in which the green pigment had entirely disappeared and the cells had become greyish-yellow in colour. In this condition they were entirely inactive and possibly not a few of them were dead. Here

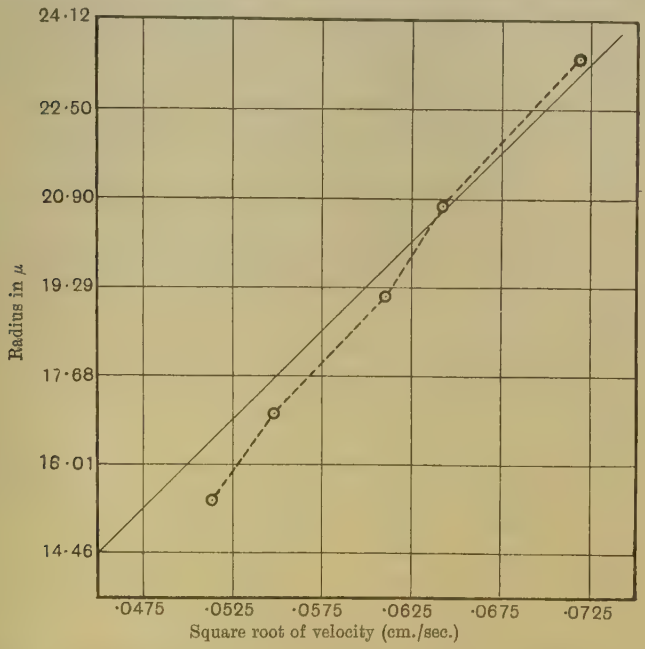

Fig. 4. The regression of velocity on diameter in the cysts of *Gonostomum* (sp.).

the density was consistently low as compared with the young cells, and its variation with the size of the cell was not nearly so marked. The average value for the density was 1·057.

When grown upon a solid substratum, the central portion of the mass frequently loses its green colour and turns yellow, but in the margin of the culture, often the colour remains a deep green for some time. A considerable number of the green and yellow cells from such a culture were tested separately and the average density of the green cells for all sizes was 1·102, while that for the yellow cells was 1·060.

Further study was made upon cells from a flask culture upon solid substratum which had developed a brownish pigment. These were kindly furnished by Dr Bristol from one of her stock cultures. She regards the cells in this state as still vigorously active. The average density for all sizes in this group was found to be 1·094.

It would thus appear that in addition to the causes indicated above as possibly explaining the decrease in density of algal cells with increase in size and age, there may also be a point in the process of maturation, following which the actual condition of the protoplasm may become an important factor in reducing this value. This would hold particularly where the environment, for some cause or other, is inimical to the progress of normal growth.

THE DENSITY OF PROTOZOAN CELLS.

In studying the density of protozoan cells, because of the difficulty of paralysing or killing the active forms so that their bodies would remain unaltered both in form and composition, the cysts were employed. However, where the measurements upon the density of the cystic forms have been made under certain conditions, described below, an attempt has been made to correlate this value with that of the living active form.

DENSITY OF THE CYSTS OF *GONOSTOMUM* SP.

Gonostomum sp. is one of the larger types of soil protozoa, measuring from 80–120 μ in length, 20–30 μ in breadth and 5–8 μ in thickness. Thus, in general form, it is rather slender and sometimes quite thin. Its shape, however, like most protozoa will be determined to a considerable extent by the medium upon which it is grown and particularly the nature and extent of the food supply. This same type of organism is sometimes referred to in the literature as Gastrostylus. The average diameter of the cysts, measured in the course of these studies, was found to be 39·2 μ. They are usually brownish in colour, and appear quite dense with well defined margins bounded by fairly heavy, crenate walls.

In Fig. 4 the graph shows average radius values plotted against average velocities. All the points are not located since their distribution about the main axis was found to be quite similar in nature to that noted for the alga in Fig. 2. Particular attention is called to the proximity of the three middle points to the main axis which passes through the origin since, as will be pointed out later (distribution curve, Fig. 5), the larger part of the individuals measured fell in this region. The nature of the deviation of these points from a straight line independent of the

main axis, suggests, as in the case of the algal cells, an inverse relation of density to size, though in this case the variation is much less marked.

The curve representing the actual variations in the density of the cysts is shown in Fig. 5 *A*. For convenience in comparing the density, as well as the nature of the variations between the *Gonostomum* cysts and algal cells, the curve for the latter is shown at *C*.

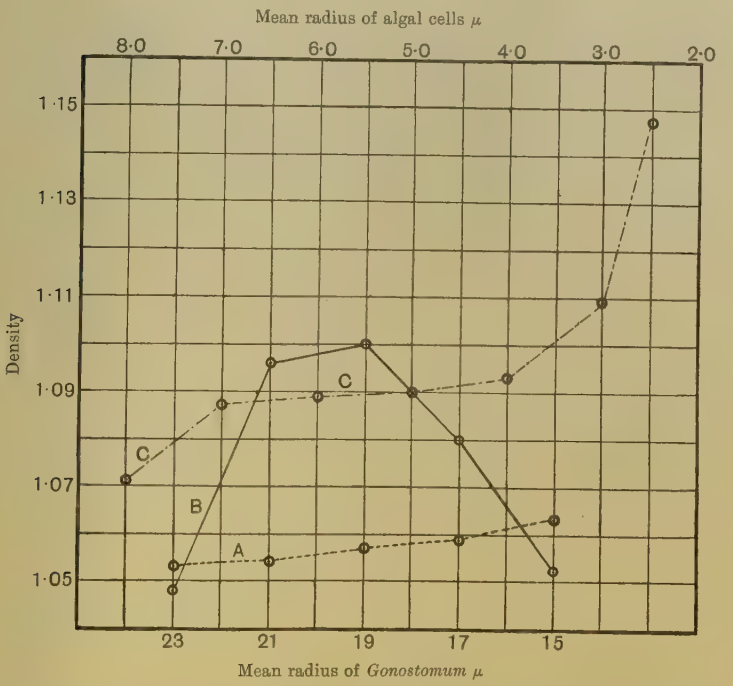

Fig. 5. The correlation of density of cysts (curve *A*) of *Gonostomum* (sp.) with diameter; (*B*) being the distribution curve of numbers measured, and (*C*) the comparative correlation curve for algae (see Fig. 2).

It is seen that the total variation in density in the case of the cysts is strikingly smaller than that for the algae. Likewise, the variation, though slight, is much more constant in the case of the cysts throughout the series. From this it would appear possible that an age factor in its relation to the maturation of the individual cyst and consequently its size and density, may be definitely correlated with this tendency. This

will be referred to again where, in the case of the young and old cysts of a certain species of *Colpoda*, it is most marked.

The distribution curve for the *Gonostomum* cysts measured is also shown in Fig. 5 *B*. Thus the three middle points whose total variation in density is but 0·005 represents 91 per cent. of the individuals examined. The average density calculated for the entire population measured is 1·057; this coincides exactly with the median value found under the apex of the distribution curve. Thus, in terms of comparative average values, the density of the algal cells is 1·089 while that of the protozoan cysts is 1·057 or about 35 per cent. less.

THE DENSITY OF CYSTS OF *COLPODA* SP. AND OF *HARTMANELLA HYALINA*.

In studying the density of the cysts of *Colpoda* sp. while no particular difference was expected from those of *Gonostomum* when mature cysts were used in both cases, the variation in size of the former with age

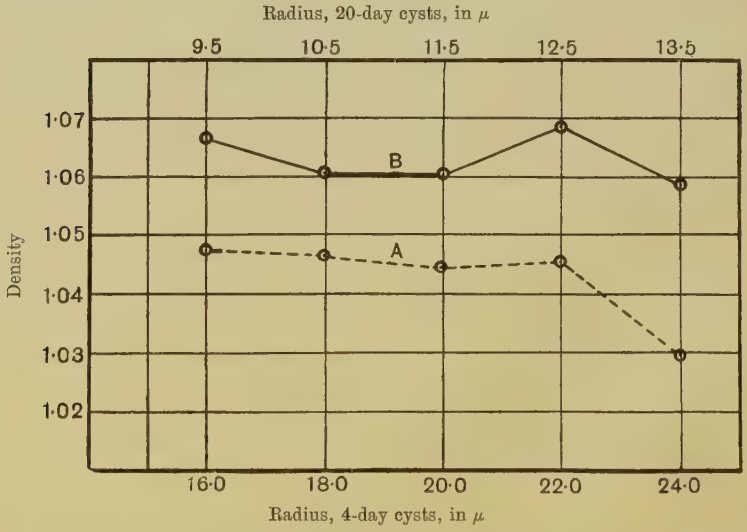

Fig. 6. The correlation of density (*A* 4-day, *B* 20-day) with the degree of maturation of cysts of *Colpoda* (sp.).

made it desirable to investigate them particularly from this standpoint. The *Colpoda* sp. used was an unusually large type, especially in the young cultures when the food material was particularly abundant.

Another point which has proved of considerable interest in the case of the young cysts is the quickness with which excystation occurs. This was emphasised upon washing a set off the plates, in order to transfer them to the tap water in which they were to be studied; upon allowing them to stand for short periods to settle between successive washings it was found that in about five hours practically all had excysted. This process did not occur in so short a time in any case with more mature cysts.

Four-day and 20-day cultures were selected for comparison. The average diameter of all the cysts measured from the former was 40·1 μ and from the latter 25·12 μ. The 4-day cysts were washed and immediately suspended in tap water and the measurements usually completed within an hour after their removal from the plate. The average individual density for each group was 1·042 and 1·061 respectively. The fluctuations in density with size as well as the relative density of the various sizes between the two groups is shown in the graph of Fig. 6.

It is evident from the figure that with the progress of maturation, the density has increased very materially.

DRY WEIGHT OF CYSTS.

In order to analyse further the nature of this loss in weight of the cysts during maturation, an attempt has been made, upon the basis of their observed density, to apply the formula of Hehner and Richmond to the estimation of the actual dry matter which the cysts contain. This formula

$$T = 0{\cdot}2625\,\frac{G}{D} + 1{\cdot}2F,$$

where T represents total solids, D the specific gravity, $G = D - 1$ and $F =$ the percentage of fat, has been developed particularly for the estimation of the total solids in milk when the density of the whole liquid and the fat content are known. While the involved assumption that the difference in the specific gravity of the milk serum and water is directly proportional to the percentage of solids in solution is not always strictly correct, Wiley (6) states that the error arising from this source, even in extreme cases, is never more than 0·5 per cent.

Neglecting the small amount of fats and waxes which the cysts probably contain, it is found that, by the application of the formula, the calculated dry matter in the young cysts amounts to 10·6 per cent. while that for the mature cysts is 15·1. Collecting together into a single

table the data that have been obtained upon the two sets of cysts by the above method we have:

	Cysts of *Colpoda* sp.	
	Young (4-day)	Mature (20-day)
Average diameter	40·1	25·12
Average density	1·042	1·061
Weight (mg.) of individual cyst ...	·00003518	·00000881
Percentage dry matter	10·6	15·1
Dry matter (mg.) per cyst ...	·00000373	·00000133
Water (mg.) per cyst	·000003145	·00000748
Total loss in dry matter		·00000240
Total loss in water		·00002397
Water percentage of total loss ...		90·9
Dry matter percentage of total loss		9·1

From this, it would appear that while the cysts lose a considerable portion of their dry matter (64 per cent.) during maturation, the loss of water (76·2) is considerably greater through the same period. With regard to the relative loss of the two constituents, it is found that 90·9 per cent. of the total loss in weight is due to water and 9·1 per cent. to dry matter.

If the value 6·8 per cent. of neutral fat for the protoplasm of Plasmodium, as found by Lepeschkin(5), be tentatively adopted in these calculations as the content of analogous materials in these cystic bodies, it will only increase the calculated dry matter in either case by a little over 1 per cent. The water content of the protoplasm of Plasmodium was given as 17·4 per cent.

Calculating the dry matter for the algal cells in the same manner upon the basis of average density, it will be found to be 23·43 per cent. while that for the *Gonostomum* cysts is 14·15 per cent.

THE DENSITY OF ACTIVE PROTOZOA.

With regard to the density of the active organisms, due to the difficulty of killing or paralysing them as well as measuring and calculating the volume of their irregular bodies, as was pointed out above, the nearest approach that has been made to such a value has been in the use of the young cysts discussed above. In a number of cases while their measurement was in progress the protoplasm of these cysts began to rotate, an activity that is usually found to precede excystment. In some cases shortly after the measurement of the cyst was completed it divided into two or four young organisms which after

some difficulty finally became separated and swam away as free individuals. Thus instead of trying to measure irregular and active forms, which in no way now known can be caused to fall inertly and uniformly through the suspending liquid, it is much simpler to measure the cystic form immediately before excystation occurs. Since the bodies of the young animals constitute the entire mass of the mother cyst it would seem that the density of the latter would represent fairly closely the density of the daughter cells at the time of their separation.

Since there is no consistent variation in the densities of those cells which give evidence of excysting or did excyst shortly after their observation was completed as compared to the other cells of the same age, it is thought that the average density (1·042) presented above for the entire group of 4-day cysts may represent fairly closely the density of the active organisms excysting therefrom, directly the process is completed. While there may be a slight difference in density in young and freshly excysted animals as compared to older individuals, it is thought that this difference will not be found to be great. This, however, is a matter which cannot be discussed upon the basis of the data available at the present time.

THE DENSITY OF *HARTMANELLA HYALINA* CYSTS.

The measurements so far carried out upon the density of *Hartmanella* cysts have not been entirely satisfactory. Therefore the average density of 1·084 as calculated from the results of some fifteen measurements will be presented as tentative until the value can be worked out upon the basis of larger numbers. With regard to the relation of size to density, the latter increases directly with size, the range being from 1·064 to 1·104.

These studies were carried out in the Department of Protozoology at Rothamsted, and it is a pleasure to thank Mr Cutler for his critical interest during the progress of the work and during the preparation of the manuscript.

SUMMARY.

1. By measuring the rate of fall of certain unicellular organisms of known diameter under appropriate conditions, their density has been calculated by the formula of Stokes.

2. According to this method the average density of algal cells studied is 1·098 and that for the cysts of *Gonostomum* sp. 1·057.

3. The density of the algal cells was found to vary greatly between the larger and smaller sizes, while that for cells intermediate was fairly constant. In the case of protozoan cysts the total variations in average density was much less marked.

4. It was found that during maturation, the cysts of a certain species of *Colpoda* decreased to one fourth their original volume, while their average density increased from 1·04 to 1·06.

5. Since many of the 4-day cysts of *Colpoda* sp. excysted during or shortly after the measurement of their density, the average density, 1·042, for this group is thought to represent quite accurately the density of the young animals in this early stage.

6. By the application of the formula of Hehner and Richmond to the density values so obtained, a tentative value has been derived for the actual dry matter of the cells studied.

7. It has been found by calculation upon this basis that the dry matter of the young cysts (4-day) of *Colpoda* sp. amounts to 10·6 per cent. while at the later stage (20-day) it is 15·1 per cent.

8. It appears that 64·3 per cent. of the total dry matter of the cyst and 76·2 per cent. of the water content are lost during maturation. With regard to the total percentage of loss in terms of either constituent, it has been found that water represents 90·9 per cent. and dry matter 9·1 per cent. of the total loss respectively.

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THE MULBERRY "BLIGHT" IN BRITAIN

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(With Plate II.)

A disease of mulberry trees, recorded for this country in the present note, first came under the writer's notice as long ago as 1914. From material obtained in 1915 a white bacterium was isolated which, by inoculations made in the spring of the following year, was shown to be the organism causing the characteristic lesions. Pressure of other work at the time prevented a study of the various physiological reactions necessary for the complete identification of the organism but from the general symptoms of the disease it was suspected that the organism was *Bacterium mori* which has been shown by Dr Erwin F. Smith to be the cause of mulberry "blight" in France and in America. The organism was kept under culture and, as the occurrence in Britain of a pathogenic mulberry bacterium remained unrecorded, it was considered desirable that its identity should be determined. Recently the necessary tests have been carried out and as they confirm the previous impression that the organism is *Bacterium mori* a brief description of "Mulberry Blight" as occurring in this country may be of interest.

Early in July, 1914, diseased mulberry shoots were sent from Bognor, Sussex, to Wye College for examination. The shoots showed a "die-back" apparently caused by *Fusarium lateritium*, this fungus being present on cankered areas at the base of the diseased portions of the shoots[2]. Some of the leaves however showed dark spots resembling those described as produced by *Bacterium mori*, but as the main trouble appeared to be that caused by the fungus the leaf-spot was not further investigated at that time.

In August, 1915, mulberry leaves showing similar black lesions were received from a nursery in Kent. Some of the leaves had only a few small scattered spots but others were more seriously affected, the spots

[1] The inoculation experiments and most of the physiological tests were carried out at the South-Eastern Agricultural College, Wye.

[2] See *Gard. Chron.* Aug. 26, 1916, p. 95.

being more numerous and often reaching a diameter of 2 mm. individually, while coalescing spots were not infrequent. The spots themselves were angular in outline, often distinctly so, being delimited by the smaller veins which formed borders to the spots. Each black spot on these badly diseased leaves, as seen from above, was surrounded by a zone of yellowish green tissue. This zone was about 1 mm. across but gradually merged into the general green of the leaf; when several spots were in close proximity the zones became confluent, forming irregular yellowish areas containing the black spots. This pale zone was not noticeable on the lighter lower surface but it was easily seen by holding a leaf up to a window when, by transmitted light, it had a water-soaked appearance. The black spots were invariably found to be crowded with bacteria.

In the nursery the disease was chiefly on young trees which had been removed from the parent "stool" the previous year after having been layered for some five years. A close examination of these young trees showed that not only were the leaves infected but that black areas were to be found on the bark of the young shoots also. The disease had been noticed on these young trees during the previous season about three months after they were planted out. The stools (about 60 years old) from which the layered shoots had been taken also bore a number of diseased leaves but the spotting was not nearly so pronounced as on the young trees planted out. A few diseased leaves were also found on a number of well-established trees (about 20 years old) which were growing in close proximity to the young trees.

From the nature of the case it appears probable that the outbreak originated in the stool-bed, that the shoots when removed from the stools were already infected to some extent, and that the conditions obtaining when the trees were planted out favoured the spread of the disease.

The first isolation plates, prepared in 1915 from infected Kent material, produced bright yellow colonies only, but shortly afterwards specimens from Bognor yielded plates consisting of white colonies with a few yellow ones. In the following year three young trees with spotted leaves were received from the nursery referred to above and isolation plates gave rise in this instance to white colonies only, similar to those obtained from the Sussex specimens.

In 1916 a young healthy mulberry tree[1] growing in a pot in the greenhouse was inoculated with the white and with the yellow organisms.

[1] This tree was kindly presented for inoculation experiments by Mr A. E. Bunyard of Maidstone.

Drops of condensation water containing the particular organism were taken from cultures 24 hours old and placed on selected leaves. Three leaves were inoculated on the upper surface and three on the lower surface without wounding, while three others were punctured at the inoculated spots. On each leaf four drops of the inoculum were so placed as to form, roughly, the corners of a square on one side of the midrib; where a leaf was wounded control punctures (not inoculated) were made on the other side of the midrib of the same leaf.

The yellow organism produced no infection whatever. The white organism on the other hand readily produced black spots at all the inoculated punctures and in a few instances black spots appeared at the inoculated spots not punctured. On the sixth day after inoculation the spots successfully infected were seen as small water-soaked areas best observed by transmitted light; later these became black spots about 1 mm. in diameter surrounded by a yellowish zone. The black spots remained about the same size but the yellowing extended. One of these leaves was removed about five weeks after inoculation and is shown in Pl. II, fig. 2; the spots were found to be swarming with bacteria and from one of the spots the organism was re-isolated. The other infected leaves turned yellow and fell comparatively early in the season.

The two organisms were used in another experiment in which the leaves of certain branches were sprayed with suspensions of the bacteria, some of the leaves being punctured. Again the yellow organism produced no blackening and the leaves inoculated with it showed no injury apart from the actual puncturing. The white organism produced, as before, the characteristic spots on all the punctured leaves but not on those uninjured, a fact suggesting that the bacterium requires a wound through which it can gain an entrance, although in the previous experiment a few black lesions appeared at spots inoculated without puncturing. The eight infected leaves eventually turned yellow and fell prematurely.

Control branches were sprayed with sterile water and some of the leaves punctured; of some thirty punctures on these control branches blackening appeared at six, but later than on the leaves primarily inoculated and they probably became infected indirectly from the other spots.

A third inoculation experiment was carried out the same summer using the organism re-isolated from one of the leaves infected in the first experiment. Inoculated punctures again gave rise to the typical lesions with a premature fall of the infected leaves.

The inoculations were continued in 1919 on a tree (in a pot) in the

open, using a sub-culture of the original white organism. Again, inoculated punctures readily became infected and from one of the spots the bacterium was re-isolated as shown by successful inoculations carried out in 1921 and 1922 using this re-isolated line.

In these later experiments it was found that inoculated punctures made on the internodes of the shoots gave rise to lesions resulting in some cases in the death of the portions terminal to the punctures. Thus in each of seven out of ten inoculated shoots the whole of the internode on which the inoculation was made became blackened and that portion of the shoot terminal to the wound died, while on each of the other three there was a black longitudinal lesion 3 to 10 mm. in length.

The experiments show clearly that the black lesions on the leaves and shoots are caused by the white bacterium used in the inoculations; the yellow organism is obviously a saprophyte in the spots and after the earlier inoculation experiments it was not studied further. That a yellow organism is frequently found associated with *Bacterium mori* has been commented on by Smith (4). Italian workers have attributed this or a similar disease to a yellow bacillus.

The morphology and physiology of the white organism conform closely with the descriptions of *Bacterium mori* given by Erwin F. Smith (3) who was the first to describe it fully enough for identification, and by Doidge (1) who, in a paper recording the occurrence of mulberry "blight" in South Africa, gives a history of the disease and a description of the organism. As Dr Doidge's article appeared in this *Journal* it is unnecessary to deal with these points in detail, especially as they are also easily accessible in a recent book by Dr Erwin Smith (6). Its growth on nutrient agar, nutrient gelatine and potato plugs is as described by the authors mentioned, as is also its behaviour in nutrient broth, Uschinsky's solution, milk and litmus milk; it does not liquefy gelatine or solidified blood serum. It is strongly aerobic as shown by the fact that, in the writer's experiments, in fermentation tubes containing peptone solution with either glucose, glycerine, lactose, maltose, mannite or saccharose the liquid in the closed limb remained clear for about three months; in fact turbidity did not extend into the closed limb until evaporation had proceeded so far that a bubble of air appeared in that limb.

In broth containing 6 per cent. sodium chloride the organism made a little stringy growth; an examination of the resulting sediment showed it to consist of filamentous involution forms. The filaments were so intertwined that they were not easily measured but in some cases where they were floating free they could be measured and were found to be

frequently over 100μ in length; the longest measured was 180μ long. Intercalary or, more often, terminal on the filaments were fusiform or spherical bodies, the largest seen being 8μ in diameter. Both Smith and Doidge found involution forms in broth containing 6 per cent. sodium chloride but did not describe them.

A little stringy growth was also obtained in bouillon containing 6·5 per cent. sodium chloride but there was no growth with 7 per cent. Doidge records feeble growth in broth containing 6·5–7·5 per cent.

There was no growth in Cohn's solution—Doidge records "no growth" and Smith "no growth or only very slight." No indol could be detected in peptone water cultures and there was no reduction of nitrate to nitrite in nutrient broth containing 0·5 per cent. potassium nitrate.

The organism is very sensitive to sunlight. In the writer's experiments plates were poured and then exposed (bottom upwards on ice) to the midday sun in November, one half of each plate being shaded with black paper. The shaded half in each case gave rise to many colonies; on the unshaded half exposure for 30 minutes killed 90 per cent. of the organisms on one plate and all of them on another; on plates exposed for one hour no colonies appeared except on the shaded half.

It is resistant to desiccation; coverglass smears were not killed by several days' drying at room temperature; on one occasion a coverglass smear was found to be viable after drying for 30 days.

The thermal death point is round about 51° C.; Smith gives it as about 51·5° C. while Doidge found 50° C. to be sufficient. The writer found that temperatures of 48° to 50° for 10 minutes retarded the appearance of turbidity in inoculated tubes while a temperature of 51° C. resulted in no growth whatever.

As in Doidge's and in Smith's later trials the rods were found to have several polar flagella; by staining with Muir's Pitfield method and particularly by Moore's modification of Loeffler's one to six flagella were demonstrated. The method recommended by Plimmer and Paine[1] (2) also proved a quick and easy method of staining the flagella of this organism.

Control. There appears to be no satisfactory method known of controlling this disease on old trees especially if the trouble is of old standing, and in the literature consulted the case of nursery stocks is not discussed. It is obvious however that healthy "stools" should be used for propagation and seriously affected stools should be destroyed by burning. If a stool is slightly affected diseased layers should be cut out and burnt;

[1] This test was carried out by Mr R. V. Harris, working in the Pathological Laboratory at the East Malling Research Station.

on no account should layers showing black lesions on the leaves or stems be planted up. Young trees should not be bedded out in the neighbourhood of established mulberry trees, particularly if the latter are known or suspected to have the disease.

SUMMARY.

1. The occurrence of bacterial Mulberry "Blight" in Sussex and Kent is here recorded.

2. It has been found on well-established trees, on "stools," and on young trees during the first and second years after removal from the stools.

3. The bacterium causing the disease has been isolated from lesions on the leaves and this has been found to conform to descriptions of *Bacterium mori* Boyer and Lambert.

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EXPLANATION OF PLATE II

Fig. 1. Branch of naturally infected mulberry tree; in centre a healthy twig, on right and left twigs with diseased leaves which are generally paler than normal leaves but have black bacterial spots.

Fig. 2. Leaf inoculated from a pure culture of *Bacterium mori*; on the right inoculated punctures have given rise to black spots with a pale zone round each; on the left of the midrib are four control punctures.

Fig. 3. Leaf inoculated by spraying with a suspension of the organism; black spots have arisen only at the punctures.

(*Received March 18th,* 1924.)

Fig. 1

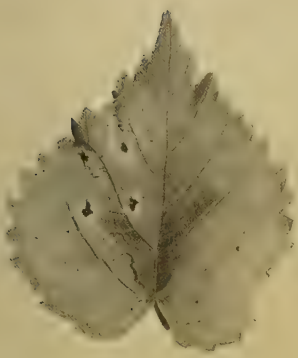

Fig. 2

Fig. 3

WORMALD.—The Mulberry "Blight" in Britain. (pp. 169—174.)

ON *CHLOROPS TAENIOPUS* MEIG. (THE GOUT FLY OF BARLEY)

By J. G. H. FREW, M.Sc. (Birmingham),

Ministry of Agriculture Research Scholar.

(*From the Entomological Dept., Rothamsted Experimental Station.*)

(With 4 Text-figures and Plates III and IV.)

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INTRODUCTION.

The following paper deals with observations and experiments upon the life-history of *Chlorops taeniopus*; the work has been carried out at Rothamsted Experimental Station during the past two years under the direction of Dr A. D. Imms. Wherever possible, I have tried to put field observations upon a strictly numerical basis, and the advantages of this method have been very great. It is impossible to publish the mass of data accumulated, but in most cases I have indicated in the text the type and the extent of the data upon which my conclusions are based. Several interesting lines of work, particularly in regard to the habits and tropisms of the imagines, have scarcely been touched upon: attention

has been concentrated mainly upon those aspects of the problem which seemed to offer the greatest probability of indicating control measures.

I have not given a complete list of the literature relating to *Chlorops taeniopus* because the position of the whole question up to 1870 is admirably summarised by Nowicki (3). He refers to about 40 different papers (some of which I have been unable to obtain) and gives many new observations of his own and of a farmer who collaborated with him. Since 1871 practically no important new work has been published. The best account of the pest in England is that by Ormerod (4). Fulmek's paper (2) contains a few new observations mainly with regard to the winter generation.

I wish here to express my sincere thanks to Dr A. D. Imms for much valuable advice during the progress of this investigation and for his constant willingness to discuss difficult points which have arisen. This opportunity is also taken to acknowledge the valuable assistance given in various ways by Dr W. E. Brenchley, Miss W. A. Mackenzie and Messrs J. E. Collin, S. J. K. Eames, R. A. Fisher, H. V. Garner, E. Somerfield and W. Young.

LIFE-HISTORY.

Chlorops taeniopus has two generations per year, a winter and a summer, the former covering much the longer period of the two. A typical life cycle is as follows. The flies emerge from their winter host (couch grass[1]) towards the end of May and lay their eggs on the leaves of the shoots of spring barley. After about eight days the eggs hatch and the young first instar larvae make their way into the centre of the shoot to the young ear. Starting from the apex of this they eat their way down one side of the ear and down the internode below it, gnawing a dark brown food groove. When mature the larva turns round, still within the shoot, until its head is directed upwards. It then ascends a short distance and pupates within the host shoot. Pupation occurs during July and the pupal period is about 36 days. The majority of the flies emerge during August but a few may be much later. These flies lay their eggs on couch grass and the larvae pass the whole of the winter head downwards in the grass shoots. Towards the end of February in the following year the mature larvae turn round within the shoots until the head is directed upwards and pupate, within the host shoots, during the early part of March. The pupal period is slightly over 60 days and the flies emerge during the end of May and the beginning of June.

[1] *Agropyrum repens.*

The different stages of the life cycle may now be examined in more detail.

The eggs are laid singly on the leaves of the host plant—usually on the upper side of the lamina fairly close to the ligule, but occasionally far from the ligule, near the tip of the leaf. They are also fairly frequently laid on the under surface of the leaf; and rarely, on the sheathing part of the leaf, altogether below the ligule.

The following figures give the relative numbers of eggs found on the upper surface of the lamina, its lower surface, and on the leaf sheath. The figures are taken from certain counts of eggs on spring barley in 1923 which will be referred to later, and as they represent the results of an examination of over 3000 shoots they are probably fairly exact:

Upper side of lamina	Lower side of lamina	Sheathing part of leaf
360 (65·1 %)	161 (29·1 %)	32 (5·8 %)

Out of a total of 490 shoots bearing eggs only 53 (10·8 per cent.) bore two eggs and only 5 (1 per cent.) bore 3 eggs, all the remainder bearing only one egg per shoot, no shoot being found bearing more than three eggs. Out of a total of 508 leaf laminae bearing eggs only 11 (2·14 per cent.) bore two eggs, and only one bore three eggs.

The eggs always lie between two adjacent longitudinal ridges of the leaf surface. One end of the egg, the anterior, is slightly dorso-ventrally flattened and this end is always directed towards the apex of the leaf. The egg is fastened down to the leaf surface by a small amount of a colourless cementing material. Upon hatching the anterior end of the egg splits into dorsal and ventral halves. The appearance of the egg after hatching is extremely variable; the two halves of the split anterior end may close together so perfectly after hatching that only by microscopic examination can one be certain whether or not it has hatched, as the egg shell itself is quite opaque both before and after hatching and does not change its white colour. Frequently, however, the two halves of the anterior end of the egg remain separate after hatching, or the empty egg may crumple up considerably. These differences after hatching are probably due to differences in atmospheric humidity.

The eggs do not fall from the leaves immediately after hatching but remain for some time, being probably eventually washed off by the rain. Possibly the growth of the leaf tissue after the egg is laid gradually cracks and loosens the cement which fastens the egg to the leaf.

During the latter half of August 1922, ten observations were made on

the period of incubation of eggs laid at this time; each of the ten observations gave either 8 or 9 days. No careful observations have been made on the period of incubation of eggs laid in early summer (*i.e.* those laid by flies emerging from the winter generation) but it is probably little, if any, different from the period given above. The incubation period probably varies slightly with low or high temperature.

Larval Stages.

The morphology of the larval stages has been described elsewhere (1). The actual hatching of the egg has unfortunately never been observed so that the actual penetration of the first instar larva has not been seen. It is almost certain that the eggs normally hatch some time during the night —probably in the early morning hours, and although various conditions of humidity, etc., have been tried I have not succeeded in the laboratory in getting eggs to hatch during the day time.

It also seems probable that the first instar larvae must penetrate into the plant during the night. On several occasions small pieces of leaves bearing eggs nearly due to hatch have been left in glass tubes overnight. The eggs have hatched and the first instar larvae easily found in the morning. Not one of these larvae, however, has ever penetrated into a plant upon which it was placed in the day time.

After numerous dissections of barley shoots containing first and second instars of *Chlorops* the mode of penetration of the larva seems to me to be as follows. The larva upon emerging from the egg has its head directed towards the apex of the leaf. It must therefore turn round before entering the shoot. Supposing the egg to have been on the upper surface of the leaf the larva makes its way down to the ligule, crawls round between the ligule and the lamina until it comes to the slit between the overlapping edges of the sheathing portion of the leaf, and through this slit it enters the shoot. It then usually works inwards and downwards in a spiral manner through the slits between the edges of successive sheathing leaves until it reaches the centre of the shoot. Instances have been seen where the larva has bored directly through one or more of the inner sheathing leaves on its way to the centre of the shoot. Nowicki (3) suggested that the larva might possibly penetrate into the plant by boring through the ligule, but I think this very improbable. Although, as stated above, the first instar larva does occasionally bore through the tender inner sheathing leaves, it is not, I imagine, sufficiently strong to bore through the relatively tough and membranous ligule of one of the outer leaves. The larvae from eggs laid on the lower side of the lamina,

or on the sheathing part of the leaf, similarly enter the shoot between the approximated edges of the sheathing leaves.

The first larval instar is apneustic (1) and cannot therefore be expected to have a long existence. As I have not been able to observe the actual penetration of a first instar larva into a shoot I cannot state definitely the duration of this phase, but it is almost certainly less than 48 hours. First instar larvae which have hatched out one night have been found to have migrated far into the shoot when this was dissected next morning. The first instar larva is, in fact, essentially a migratory phase. It undoubtedly feeds to some extent, as green chlorophyll coloured matter can be found in its gut, and also it grows somewhat before moulting into the second instar. I have, however, never been able to find any marks due to the feeding of the first instar, except in the comparatively rare cases where it has bored through one of the inner sheathing leaves. The primarily migratory nature of the first larval stage is a common feature among parasitic Cyclorrhaphous larvae, or indeed among animal parasites in general.

In a typical case of infection by the summer generation the first instar larva reaches the apex of the tender young ear before changing into the second instar; and in a typical case of winter attack it attains almost to the base of the shoot. This point as regards the summer attack will be discussed in detail later. Deformation of the plant (see below) is not apparent until the larva reaches the second instar. This does not mean that deformation may not be initiated by the first instar, because the deformation would necessarily take some time to become apparent after the impulse to deformation had been given, and the life of the first instar is only of short duration.

The feeding habits of the second and third larval instars will be found discussed below in connection with their effect on the plant, and to avoid repetition a few general points only are considered here. The first and second instar larvae are subject to a definite tropism causing the first instar to migrate towards the base of the leaf upon which it hatches, and causing both first and second instar larvae to move downwards when within the shoot. The tropism is probably primarily a food one which has become an ingrained larval habit. In the majority of cases this habit is beneficial and leads the larva to its food, but in certain rather rare conditions, which will be discussed in connection with the effect of manures on infestation, the habit acts against the larva and destroys it by leading it away from suitable food. That the tropism is not a gravitational one is shown by the fact that larvae hatching out from the egg

will enter the shoot upon which they hatch even if it is placed in a horizontal position[1], and if the tropism is not gravitational for the first instar it is probably not so for the second and third instars. What little evidence I have upon these points is quite fortuitous and derived from data obtained to determine entirely different questions.

In the winter generation the third instar larva does not usually move downwards at all, in the summer generation it usually does so (see below, Effect on the Plant). In both cases the mature larva, which is head downwards during the greater part of its life, turns round a short time prior to pupation so that its head is directed upwards. This is almost certainly not an effect of gravity because if mature larvae are removed from their host plant and placed upon moist filter paper in tubes, they will pupate horizontally, head up, or head down, and normal healthy flies will emerge. It cannot be due to a suddenly acquired positive heliotropism because in the great majority of cases no light can penetrate to the larva. It is undoubtedly connected in some way with a change in the physiological state of the larva when it becomes fully fed. In all cases after turning round the larva migrates upwards a short distance before pupating and it may be a pressure stimulus which causes the larva to seek a relatively spacious site for its pupation. Instances have been seen of puparia which failed to develop because pupation occurred in too restricted a space and the puparium was consequently flattened. Apart from the probable necessity of finding space for pupation, if a larva pupated head downwards the fly, when it emerged, would very probably fail to escape from the shoot.

In the case of the summer attack (see below) pupation may occur in the food groove on the ear bearing internode, on the ear, or above the ear. It was thought that the distance of upward migration of the larvae might be constant, within small limits, for all larvae and that the position of pupation with regard to the ear depended upon the dimensions of the shoot. This is not so, as measurements of the length of migration were made on 50 shoots and were found to vary from $\frac{3}{4}$ in. to $6\frac{1}{4}$ ins. The distance of upward migration of the winter larvae was not measured, but the attacked shoots themselves are sometimes less than 1 in. in length and the migration therefore necessarily restricted. Larvae never leave the host plant to pupate either in the winter or summer generation.

[1] Unfortunately the experiment of inverting the shoot was not carried out. The horizontal position does not give quite definite proof that this tropism is not to some extent gravitational.

EFFECT ON THE PLANT.

(Plates III and IV.)

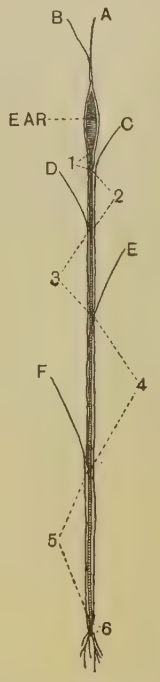
The general effect of an attack of either the winter or summer generation of gout fly is to produce stunting and thickening of the attacked shoot with considerable increase in the breadth, and moderate increase in the thickness of the leaves. The deformation produced by the winter attack and by the summer attack have usually been described as entirely different in appearance and nature. While this is incorrect if all forms of the damage are considered one can nevertheless distinguish quite sharply the typical form of winter damage and the typical form of summer damage, and this is the most convenient way of describing the various intermediate forms. For the sake of convenience barley is considered as the host for both winter and summer generations, although couch grass is a far more usual host for the winter generation.

Typical winter form (on winter barley). (Plate III, fig. 1.)

Attacked shoots are short and very thick, with broad, short and somewhat thickened leaves having a healthy green colour. No stem is recognisable, being so extremely stunted that the leaf sheaths all appear to arise at practically one point at ground level. The thickness of the shoot is due to the thickness and breadth of the separate leaves which surround each other. The edges of the leaf laminae are usually considerably crinkled and the leaf veins are usually wavy instead of straight. The laminae of the more central leaves are curled in a spiral manner; the outer ones less so or not at all. The tips of the leaves are frequently dead and frayed out. This condition of the plant makes its appearance about the beginning of December (earlier or later according to the date of oviposition upon it) and is maintained without change or growth all through the winter until towards the beginning of March, when the larvae commence to pupate. As soon as a larva pupates the attacked shoot commences to wither and die, and decay soon sets in. It seems probable that the feeding larva secretes some substance (probably from its salivary glands) which maintains the attacked shoot in the green and sappy condition necessary for the nutrition of the larva.

Typical summer form (on spring barley). (Plate IV, figs. 1 and 2.)

While the great majority of attacked winter shoots are of the type described above, the type of distortion produced by the summer attack varies very greatly and depends entirely upon the stage of growth of the shoot when attacked, while the degree of distortion produced depends upon the rapidity with which growth is progressing at the time of the attack. The typical form will show a stem of two or three internodes, and then a thick upper region which consists of the ear completely enclosed in its sheathing leaf from which it never escapes. It will be convenient to define the plant at the time of commencement of the attack, and then describe separately the effect of the attack upon stem, leaves and ear.



Text-fig. 1. Diagram of shoot of barley. 1. The ear-bearing internode. 2–6. The lower internodes of the stem. *A–F*. The leaves.

At the time when the egg causing the attack was laid the plant may be supposed to have been as represented diagrammatically in Text-fig. 1.

At the base there is the usual very short internode which has entirely finished its growth (6). Above this there are two internodes 5 and 4 which are well grown and have finished their growth. Internodes 3 and 2 are still undergoing active growth as are also the ear and the very short ear-bearing internode 1. Leaves *A*, *B* and *C* which belong respectively to internodes 1, 2 and 3 may also be supposed to be in active growth whereas leaves *D*, *E* and *F* belonging to internodes 4, 5 and 6 may be supposed to have finished their growth.

The effect of the larva on the plant is to cause an apparently almost immediate cessation of growth in length; internodes 4, 5 and 6 having already completed their growth are not affected in any way. The larva as it migrates downwards, eats away the young grain along one side of the ear, and then passes on to the ear-bearing internode (1) and eats a food groove down one side of this. The food groove increases in size and depth as it passes downwards, owing to the growth of the larva; it ends at the base of the ear-bearing internode. When about to pupate the larva

turns round in its groove and migrates upwards a longer or shorter distance before pupating, travelling in the groove which it has already made. It is difficult to be absolutely certain whether feeding does or does not go on during this upward migration, but probably it does in some cases as a few instances of ear-bearing internodes have been seen with two food grooves—one which is narrow and shallow above and gradually becomes more extensive below and is therefore the true food groove of the downwardly migrating larva, and another shallow and broad groove branching off from some point of the former. This latter groove is made on the upward migration by a larva which leaves its old food groove. In any case the feeding on the upward migration is very slight in amount.

Only those leaves which are in active growth when the attack commences are deformed in consequence of the attack, and the leaves in the typical form of attack are not nearly so greatly deformed as those of some of the less typical forms to be described below. The reason for this is simply that in the typical form all the leaves, even (A) in the plant under consideration, have already completed most of their growth when the attack starts. The visible part of (A) is usually somewhat twisted and one-half of the lamina is often very much narrower and less developed than the other. This is, I believe, due to one side of the base of the leaf having been slightly injured by the larva. Leaf (B) shows rather less distortion and leaf (C) less still. (Pl. IV, fig. 3.)

As the larva eats its way down one side of the ear it completely destroys the rudiments of grain on that side. The effect upon the grain on the undamaged side of the ear varies considerably: sometimes the grains on this side are apparently sound, sometimes apparently sound but destitute of awns, sometimes very badly shrunken. In spite of careful measurements of large numbers of attacked shoots, I can offer no definite explanation of the above variation though I believe it is probably correlated with the state of the grain on the undamaged side when the larva commenced feeding on the ear-bearing internode, and with the amount of damage done to the ear-bearing internode and the consequent disturbance of the food supply to the ear.

Up to the time of pupation the parts of the plant upon which the larva is feeding tend to remain green and sappy though this effect is not so marked as it is for the winter generation. Very shortly after pupation the ear-bearing internode becomes hard and brittle and any grains on the ear commence to set and ripen. They cannot, of course, ripen properly except in those exceptional cases where the ear succeeds in partially escaping from the sheathing leaf.

Variations in the typical form of summer attack have to do mainly with the ear and the ear-bearing internode. The degree of stunting of the internodes below the ear-bearing internode varies, of course, considerably, according to their state of growth at the time when growth was inhibited by the larval attack; the length of the ear-bearing internode and the size of the ear also varies considerably for the same reason.

Besides being stunted the ear-bearing internode may or may not be considerably thickened; it is also frequently waxy and twisted instead of straight. Occasionally the food groove does not extend to the base of the ear-bearing internode, and in three cases out of many hundreds examined, the food groove extended below the ear-bearing internode on to the stunted internode below it. The damage done to the ear varies considerably according to the stage of growth of the ear when attacked; if it was very young not a vestige of grain may develop on either side. Occasionally an ear which was well grown when attacked and is consequently not very badly damaged, will partially escape from the sheathing leaf and a few grains will ripen.

Certain special cases of damage due to the summer generation will be considered later in connection with the action of certain manures in reducing the infestation of a crop by gout fly.

Atypical forms of attacked shoots.

(*a*) *Winter forms.* When winter barley is harvested and the land left to clover many self sown plants and late tillers of barley will spring up and some of these will form a definite ear before growth ceases in the autumn. Such eared shoots may serve as winter hosts for *Chlorops* and the damage resembles precisely the typical form of summer damage.

Attacked couch grass (Pl. III, fig. 2) usually resembles the typical winter form of attacked barley; sometimes, however, a stem of couch grass with two or three internodes is attacked. In this case the basal internodes are entirely unaffected while the apical part which is affected resembles in all respects the typical winter form, except that it arises from a long stem.

(*b*) *Summer forms.* The typical form of summer damage is produced on shoots which are fairly well grown and have a moderately well developed ear when the attack commences. If barley is late sown, or if its early growth is checked, many shoots will be attacked when practically no stem formation has occurred and when the ear is still very small. Such an attacked shoot will resemble precisely the typical winter form; the ear will be entirely destroyed and no stem formation will occur

(Pl. IV, fig. 3). There is every gradation between this form and the typical summer form. Thus an attacked shoot may be like the winter form except that it arises from one, or two, or three, short basal internodes (Pl. IV, figs. 4 and 5); it may contain a small badly damaged ear or the ear may be entirely destroyed. The form of summer attack on couch grass is the same as that of the winter form; there is no form on couch grass resembling the typical summer form on barley.

The damage on wheat, both of the winter and summer generations, resembles that on barley except that so far no summer type damage has been found on wheat in winter, and it probably does not occur as the wheat harvest is too late to allow self-sown wheat to form ears before winter.

For practical purposes a shoot of barley attacked by gout fly may be considered as totally destroyed, but it is important to discover whether the effect ends there, or whether an attack upon one shoot of a plant has any bad effect upon the yield of other non-attacked shoots of the same plant. It may be said definitely that there is no visible effect. In 1922 several small experimental plots were set up. When harvested the plants were pulled up by the roots. The length of straw of every shoot and the number of sound and unsound grains in every ear were then obtained. This body of data seems to settle quite definitely that if one shoot of a plant is attacked, the other shoots are not thereby affected in any way.

The distortion of the plant produced by *Chlorops taeniopus* is in several respects extremely interesting. Unfortunately I have not as yet found time to examine sections of the plant tissue in order to determine the precise nature of the distortion as regards the individual cells. The malformation caused by *Chlorops* is, as far as I have seen, quite unique amongst grass feeding larvae, and it is unique in this respect; that the *Chlorops* larva produces distortion of parts of the plant with which it is not actually in contact, which it does not damage and which are below the region of damage and whose food supply cannot therefore be affected. This fact can be made out in almost any "gouty" shoot—with difficulty in some of the winter type damage—but is most clearly seen in some of the non-typical forms of summer damage on barley where, as already mentioned, the degree of distortion of the leaves is considerably greater than in the typical form. In such a shoot one may find a leaf arising from a node separated from the region of actual damage by one, two or even three internodes, and yet showing distinct and often very prominent signs of deformation. It is, in fact, obvious, that any region which is

actively growing when the larva commences feeding is affected, and the more active its growth at the time, the greater the distortion produced. As a general rule the younger parts are the most rapidly growing parts but there is probably one exception to this. The apical or central leaf of a shoot suffering from either the typical form of winter attack or a non-typical summer attack is very rarely as much distorted as the two leaves next below it, which are usually the most distorted leaves on the plant; it ceases to grow in length and usually becomes spirally curled but it does not become noticeably thick or broad. This is quite explainable if the growth of the leaves follows the usual growth curve which is typical for living individuals and parts of living individuals, *i.e.* rate of growth starts slowly, attains a maximum and then diminishes. The central leaf, being the youngest, will probably not have attained its maximum rate of growth when the deformative influence sets in, whereas the two leaves below it will more probably be nearly at their maximum growth-rate. In the case of the typical summer form, the attack takes place when the plant is in a more advanced stage of growth, and in this case it is the apical leaf which is nearest its maximum growth-rate—very frequently even the apical leaf has apparently passed the maximum growth-period and its growth-rate is declining when the attack takes place; hence the relatively slight distortion of the leaves in this form. I cannot at present say whether the increase in breadth and thickness of the leaves is due to increase in cell size or cell numbers. Badly deformed leaves undoubtedly give the impression of being in a state of excessive turgidity and on general grounds it seems that increase in cell size is more likely than increase in cell numbers. It has been stated above that the commencement of larval feeding inhibits growth in length. This is not strictly accurate; there must be a slight amount of irregular growth in length of the leaves to cause the twisting of their laminae and the puckering of their edges. With regard to the stem the attack has little effect beyond inhibiting growth in length of those internodes which are growing. Occasionally some of the internodes show slight twisting or bending (indicating the occasional survival of slight, irregular, growth) and may become slightly thickened.

It seems to me that the only way to explain the deformative influence of the larva upon parts of the plant below the actual damage is to suppose that the larva introduces some substance into the sap which is distributed throughout the plant and that it is this substance which causes the deformation of the growing parts of the plant. If this is so the substance in question is probably formed in the salivary glands of the larva. An

attempt was made to test this point by making an extract of several larvae in salt solution and injecting this into the plant. This experiment was done before the importance of the growth-rate on the resultant malformation was understood, and I now believe that the plants selected for the purpose were too old, and that the failure to produce the malformation artificially was largely a result of this. The difficulties of injecting liquids into the plant tissues are, of course, considerable, but I believe that artificial malformation would be produced if the larval salivary secretion could be introduced into the plant sap.

If a larva is removed from a shoot in which it has been feeding for some time, or if, for some reason, it dies within the shoot, growth does not recommence. In the case of a typical form of winter attack growth is, of course, impossible, because the young rudiment of stem and ear has been entirely destroyed. In the case of a typical summer attack from which the larva has been removed, this explanation does not hold good: it seems rather that growth is entirely and permanently inhibited. Certain exceptional cases, where the larva enters a shoot too far advanced in growth to allow the larva to develop, will be discussed later in connection with the action of manures and early sowing upon the infestation by gout fly.

The advantage to the larva of the deformation produced is fairly obvious, particularly in regard to the typical form of summer attack. The inhibition of growth prevents the ear-bearing internode from lifting the ear out of its sheathing leaf and so exposing the larva to the open; if this happened the larva would almost certainly fall off the internode as it has no adaptations for maintaining a firm hold upon a dry, smooth and vertical support. So long as the internode remains within its sheathing leaf the larva is kept safely pressed between the internode and the leaf. In some way, as already mentioned, the larval feeding has the effect of keeping the tissues green and sappy and therefore in a suitable condition for the larva. In the winter type of attack this latter function of the deformative influence is probably the more important, as the question of growth does not arise, the larva having destroyed the stem rudiment. It is remarkable upon how small a shoot a larva can develop. A small shoot of couch grass, of less diameter than the full grown larva, may be attacked; as the larva develops the leaves thicken, and the shoot becomes thicker and thicker and is apparently robust and flourishing although stunted. Thus winter larvae have been found in shoots of couch and wheat less than 1 in. long, but relatively enormously swollen. It seems probable that when a larva is in a very small shoot the latter is stimulated

to a degree of assimilation quite out of proportion to its size and in this way the *Chlorops* larva can develop in shoots initially smaller than could be used by a smaller larva such as *Chloropisca*. If very small shoots are attacked by *Chloropisca* or Frit they quite frequently die off before the contained larva can mature: these larvae are, however, quite active and it is possible that they can leave an unsuitable shoot and enter another. I have at all events succeeded in removing them from one shoot and transferring them on to the apical leaf of another close to the ligule, and in several cases they have entered the new shoot and completed their development there. I have never succeeded in doing this with *Chlorops* and I feel certain that it never occurs in natural conditions. Thus if the young *Chlorops* larva entered a very small shoot and killed it, the larva itself would die, and its method of using such small shoots by causing hypertrophy has opened up for the larvae a new source of host plant.

THE LIFE CYCLE IN ITS RELATION TO DIFFERENT HOST PLANTS.

	1922	1923
First puparium of winter generation found	March 2nd. 75 % had pupated on March 25th	March 8th. 25 % had pupated on April 4th
First fly emerged from winter generation	May 22nd	May 26th
Last „ „ „	June 10th	June 25th
First puparium of summer generation found	June 30th. 77 % had pupated on July 22nd	July 12th. 26 % had pupated on July 18th
First fly emerged from summer generation	July 31st	August 10th
Last „ „ „	September 29th	?

The flies emerging from the 1921–22 winter generation laid almost exclusively upon barley. For some time it was thought that couch grass did not serve as a summer host but a few damaged shoots of couch grass were found in that year, and a considerable number were found this year (1923).

Gouty wheat was only found in one field (New Zealand Field, Rothamsted) in the summer of 1922 and has not been found at all in 1923. The reason that the wheat in this field was attacked was that it made extremely poor growth during the winter and early spring and was extremely backward at the time when oviposition was taking place. In the majority of cases winter wheat is too far advanced in growth for oviposition upon it by the flies emerging in spring.

The flies emerging from the summer generation can utilise six types of host, and the type utilised depends mainly on the time at which the flies emerge. In 1922 the flies were emerging from the beginning of

August to the end of September, though the great majority emerged between Aug. 8th and Aug. 24th. The following are the different types of host plant used.

(*a*) Couch grass, which is always available and forms the chief winter host, and which is probably the only host available for the earliest emerging flies. An interesting point about the infestation of couch grass is that couch grass on an arable field, more particularly that growing on a field of roots such as swedes or turnips, appears to be far more heavily attacked than does couch grass growing among other grasses in hedge rows, etc. Possibly the attraction of the couch grass for the fly, perhaps aromatic, is swamped and lost when the couch grass is growing among other grasses.

(*b*) Late non-earbearing tillers of spring barley coming up after harvest. Although such tillers exist plentifully before harvest the fly does not appear to seek them out among the bases of standing corn and even if it did so many of these tillers would be cut down at harvest time and the larvae consequently destroyed. This type of host is therefore only of use for late emerging flies and when the harvest is late, as it was in 1922, the infestation is negligible and may be considered as non-existent.

(*c*) Self-sown barley due to grain shaken from the ears of spring barley at harvesting. This is an even later growth than the last class and can probably be entirely disregarded unless the harvest is quite exceptionally early. A small amount of infection of this type of host was found in the winter 1921–22 but none was found in the succeeding winter.

(*d*) Late tillers growing up after the harvesting of winter barley, and self-sown barley due to grain falling from the ears of winter barley during harvesting. The harvesting of winter barley being very much earlier than that of spring barley this is a much earlier type of growth than (*b*), and is a real danger, ranking next after couch grass in the number of larvae which it carries through the winter. I imagine that it is actually preferred to couch grass as a winter host but owing to the relatively small amount of winter barley grown (in this district) it is fortunately not very abundant.

Hosts in classes (*b*), (*c*) and (*d*) are only of danger when the barley is followed by a clover ley so that the barley shoots are allowed to stand all winter. If the land is ploughed in, or if the clover is cut for fodder during the winter, the larvae within the host shoots are destroyed.

(*e*) Autumn sown barley and wheat may be attacked if they are sown sufficiently early. In 1922 flies in captivity could not be induced to lay after the middle of October and it is probably safe to say that ovi-

position does not go on after that time in the open; and in any case only a relatively small number of late emerging flies will be about at this time. Therefore if winter wheat or barley are to be infected they must be sown sufficiently early to be moderately well grown plants by the middle of October. This would mean sowing in September which is a very rare occurrence. Consequently infection of this type of host is (in this district) negligible.

(*f*) There are usually numerous self-sown barley or wheat plants round a straw stack, or on a place from which a straw stack has been removed, and such shoots are frequently suitable for the winter generation. In one instance a wheat stack had apparently been removed early in the autumn. Numerous grains of wheat germinated and provided hosts for the oviposition of some of the late emerging flies from the summer generation. The shoots were extremely small when attacked but the larvae within succeeded in attaining maturity. There was no winter wheat or barley which had been sown sufficiently early for the oviposition of these flies and all the neighbouring arable fields had been ploughed in after the cereal crop harvests so that couch grass would ordinarily have been the host for these flies. The young wheat plants were, however, concentrated in one spot and their attraction to the fly was probably therefore greater than that of scattered couch grass; consequently they were heavily infected.

THE POSSIBILITY OF AN INTERMEDIATE GENERATION.

In 1922 the first fly bred out emerged on July 31st. Some of the very early emerging flies were made to oviposit on shoots of barley and couch grass to discover whether an intermediate generation of flies could be reared. The resulting larvae, however, passed the winter in the larval state and pupated next spring at about the same time as larvae derived from eggs laid some weeks later. It seems improbable therefore that an intermediate (autumn) generation normally occurs in this country, though one might possibly be produced artificially by keeping the infected plants in conditions of temperature and light approximating to the summer conditions.

HABITS OF IMAGINES; OVIPOSITION EXPERIMENTS.

The numbers of ♂s and ♀s are approximately equal. Thus of 1461 flies bred from the summer generation in 1922, 701 were ♂s and 760 were ♀s. Copulation may take place within a few hours of emergence and before the wings of the ♀ have expanded; it has never been seen to

occur before the wings of the ♂ had expanded. Oviposition sometimes commences four, but more usually five days after copulation. The flies feed readily upon sugar solution or upon umbelliferous flowers but their natural feeding habits are not known; they are almost certainly flower feeders. The flies are fairly strong on the wing but in dull weather they are extremely sluggish, not readily taking to flight, and sometimes remaining stationary for hours. In bright sunny weather they become extremely active, and it is the brighter light rather than the increased heat which causes the difference. The ♀ will lay very few eggs in dull weather.

A number of experiments were carried out upon the oviposition of the flies emerging from the 1922 summer generation. A number of facts were brought out and it was hoped to amplify and corroborate these by means of experiments on the flies emerging in the spring of 1923. These flies were rather late in emerging but the experiments might still have been carried out but for the dull weather at the time, during which the flies would not lay. The barley had been planted in pots to be ready at a definite time but the delay of the flies in ovipositing allowed the barley to reach an advanced, and therefore unfavourable stage of growth before the advent of bright weather which stimulated the oviposition of the flies. Couch grass could have been planted and the work carried out on this, but by this time it was essential to turn attention to other parts of the investigation and consequently the 1923 oviposition experiments had to be abandoned. The conclusions are therefore based entirely upon the experiments done with the flies emerging in the autumn of 1922, and a few experiments done with the flies emerging in the spring of 1922.

For most of the experiments a ♂ and a ♀ were captured on the day of emergence and tubed together, with sugar solution for food; for some experiments no food was given, but only water. After fertilisation the ♀ was removed to the oviposition chamber, the ♂ being left in the feeding tube (which was not corked but covered over with cotton gauze). The number of eggs laid was counted, when possible, every day, and the ♀ removed to fresh shoots when necessary. Some flies had filter paper moistened with sugar solution in their oviposition chambers, others had filter paper moistened with water only. For experiments with unfertilised ♀s, puparia were tubed separately and the flies bred out so that there was no possibility of pairing. In some cases the ♀s were confined in a gauze cover covering over a whole pot in which couch grass or barley was growing but in other cases the flies were confined in a lamp chimney closed with gauze at the top, and covering one or two shoots

of couch grass or barley. The following are the chief conclusions drawn from the experiments:

(1) Pairing may take place on the same day as emergence and oviposition commences sometimes four, but usually five days after pairing.

(2) The greatest number of eggs laid by a single ♀ in captivity was 147, but several ♀s laid over 100.

(3) Unfertilised ♀s commence laying 10–12 days after emergence. Fed on sugar solution they lay about 12 eggs; no unfertilised ♀ fed on water alone laid more than three eggs[1].

(4) Unfed fertilised flies lay about half as many eggs as fertilised flies fed on sugar solution.

(5) A fed and fertilised ♀ may lay up to 17 eggs in 24 hours. An unfed fertilised ♀ only lays up to 7 eggs in 24 hours.

(6) More than one act of copulation is necessary to fertilise all the eggs that a ♀ is capable of laying. A ♂ which has fertilised a ♀ once is capable of again fertilising the same or another ♀.

Eggs were laid in greater numbers and with greater rapidity by the flies confined beneath glass lamp chimneys than by those confined under gauze covers. In the former case the flies are subject to higher temperature, greater humidity, and greater light intensity than in the latter. One of the 1923 experiments which had to be abandoned was designed to disentangle roughly the relative values of these three factors.

It is a striking and important fact concerning the flies that the ovipositing instinct of the females is not perfect. They usually select shoots of a certain type of growth, *i.e.* moderately well grown but with little development of the ear. Here their instinct is seldom at fault and they do not often lay upon a shoot which is inherently unsuitable. As a general rule eggs are laid in the field upon one of the four uppermost leaves but sometimes they are laid on the very lowest leaves where there is no chance for the young larva to develop. As will be shown later there is frequently a very considerable difference between two adjacent leaves (*e.g.* the second and third from the apex) as regards the chances of success of larvae from eggs laid upon them. In such cases the fly lays almost as frequently upon the less favourable leaf as upon the more favourable one.

The length of life of the flies has been determined only for flies emerging from the summer generation. In 1922 some flies which emerged on August 5th were placed in a large glass cage with a wire gauze top and the cage was placed in an insectary facing north so that it would not be subject to excessive sun. Pieces of filter paper moistened with sugar

[1] Such eggs do not, of course, hatch.

solution were given as food. On October 10th the flies were still alive and were removed and given the opportunity of ovipositing on couch grass. (The flies had laid a considerable number of eggs on the glass sides of their cage, where they had not been provided with any host plant.) Oviposition continued up to October 13th and the eggs laid were fertile and gave rise to normal winter larvae.

On October 16th it was noticed that the flies were becoming lethargic and were crawling about the soil in the lamp chimneys in which they were placed for oviposition, instead of seeking the top. They had laid no eggs for three days. These flies were then transferred to a dark chamber containing sphagnum moss and food in the form of filter paper moistened with sugar solution, and the dark chamber was left in the insectary over winter to discover whether the flies would hibernate. When examined on June 4th, 1923, all the flies had died. This is not, of course, a proof that hibernation does not occur, but hibernation is improbable.

The length of life of flies emerging in spring probably does not usually exceed a fortnight.

EFFECT OF MANURIAL TREATMENT UPON THE INFESTATION OF BARLEY BY GOUT FLY.

It has frequently been recorded that well manured barley fields suffer less from gout fly attack than do poorly manured fields. Hoos Field on Rothamsted Experimental Farm gives an opportunity of examining this question critically because here there are permanent barley plots which have received the same manures for many years. All these barley plots are strictly comparable except for the different manurings; they are side by side on the same type of soil and are sown at the same time.

The method used for comparing the infestation of the different plots was as follows. On each plot 10 counts were made, each of 100 shoots, and the number of infested shoots in each count of 100 was noted down. This gives for each plot 10 percentage figures of infestation, and the mean of these ten values is taken as the figure of infestation for the plot. The actual damage done to the crop is in all cases slightly higher than the figure given because the counts included all shoots, and not only those which would form ears at harvest time. The counts were done in this way because they were done some time before harvest in 1922 and the distinction between a shoot which is going to produce an ear, and a shoot which is not going to do so, is not always clear cut and definite. To attempt this distinction would therefore have introduced an error

due to personal judgment and this error might not have been constant for the different plots. Before starting on the counts on the plots it was necessary to test whether in a uniform field of barley the infestation was uniform over the whole field or whether it was greatest at the edges and diminished towards the centre, the latter of which might perhaps at first sight be expected since the flies come into the field from outside and are not originally scattered all over the field. Tests on two barley fields on Rothamsted Experimental Farm (Long Hoos Field and Stackyard Field) showed, however, that the infestation was uniform, if the headlands were excluded. On the headlands the infestation was slightly higher than within the field owing to poorer cultural conditions of the headlands in general, and some parts of them in particular. The uniformity of the infestation is due to the facts that the ♀ fly is active, and may continue oviposition for over a fortnight, and the fly definitely selects shoots of a certain type of growth for its oviposition and these favourable shoots will be scattered all over the field.

In presenting results obtained from Hoos Field it will be convenient to give first the general conclusions arrived at in 1922. I do not propose to give the full data of these manurial counts as this will be found in the statistical survey of the figures by Mathetes(5). It was found that manuring had a very marked effect upon the infestation; well manured plots being markedly less infected than unmanured plots. Farmyard manure was very effective in reducing infestation; of the artificials, superphosphate and potash were the two manures having the greatest effect in reducing infestation; $(NH_4)_2SO_4$ had some effect when acting in conjunction with some manures but no effect when acting alone, or with certain other manures. A more detailed analysis is given later. The two extremes of the series in 1922 were plot 6_1 (unmanured) with an infestation of 19·1 per cent. and plot 5A (K_2SO_4 + Super. + $(NH_4)_2SO_4$) with an infestation of 4·8 per cent.

In 1922 therefore it was definitely established that manuring had an effect upon the intensity of attack of gout fly, but exactly how this effect was produced was still unknown and it was necessary to consider all the possible ways in which the manures might be acting with a view to testing these in 1923. The possibilities may immediately be divided into two classes. Considering the two plots 6_1 (19·1 per cent. infected) and 4C (complete minerals and rape cake, 5·1 per cent. infection) the possibilities are:

A. The flies lay fewer eggs per unit number of shoots on plot 4C than on plot 6_1.

B. The flies lay the same number of eggs per unit number of shoots on both plots, but a large percentage of them fail to develop on plot 4C.

To consider A first:

If fewer eggs are laid on plot 4C than on plot 6_1, there are two possible explanations:

(1) The manurial treatment forces on the plants so that by the time egg laying commences (*i.e.* early part of June) they are in an advanced stage of growth unfavourable for oviposition.

(2) The manurial treatment may in some way alter the constitution of the plant so that its attraction for the fly (possibly aromatic) becomes less. Although this has to be considered as a possibility it is undoubtedly an improbable explanation.

If B is found to be correct there are again two possibilities:

(1) The plants on 4C may develop so rapidly in growth that by the time the eggs hatch the plants may be unsuitable (*e.g.* too tough) for the young larva, or the ear may force itself out of the sheathing leaves before the young larva can attack it.

(2) It is possible that something in the constitution of the cell sap is actively injurious to the larva and causes the death of many of them in the early stages before they have had time to inhibit the growth of the plant.

It may be said at once that B (1) is essentially correct and explains most of the observed differences. A (1) is also correct and is responsible for some part of the differences. A (2) has not been tested directly but is almost certainly incorrect. An experiment comprising 70 pots containing barley under different manurial treatments was set up in the spring of 1923 in order to test B (2). All pots were confined under a large gauze cover and gout flies were introduced into the cage. Unfortunately, as already explained, the flies would not lay during the dull weather and the plants continued to grow and became unfavourable for oviposition. The experiment consequently failed and need not be described in detail. It was hoped that by taking one infected shoot every day from each type of manure and dissecting it, that the influence (if any) of the manurial treatment upon the larva itself as apart from the influence upon the plant, could be followed out. In spite of the failure of the experiment I am convinced from a careful examination of infected shoots from certain of the Hoos plots (see later) that the manures have no injurious action upon the larva except in their effect on the plant growth.

For pointing out the possibility of the following two criticisms of

the 1922 infestation counts on Hoos Field, I am indebted to Dr A. D. Imms and Mr R. A. Fisher respectively.

(1) If any manure causes, after oviposition is finished, the development of numerous late tillers, the percentage of infestation will be reduced as these will be included in the counts.

This point was tested in 1923 by counting on seven of the plots (see Table I) the number of shoots per square yard at the time of oviposition and the number per square yard at the time when the infestation counts were made. Also the number of shoots in marked strips upon certain of the plots were counted at the same time. The former is probably the more accurate test as it represents the condition of the plot in general and not of just one part of it. The results show that the infestation counts are not invalidated by the late growth of tillers. On two of the plots (4C and 5A) the surprising result was obtained by both the above methods that there were fewer shoots at the time when the infestation counts were done than there were at the time of oviposition. This must be due to the dying down of very young tillers on these plots, the small dead and shrivelled tillers being missed in the second series of counts of shoot numbers. Some were perhaps killed by insect attack (*e.g.* frit fly or *Chloropisca circumdata*). I do not think that the death of these young tillers on 4C and 5A will affect the validity of the infestation counts (or rather the conclusions to be drawn from these—they certainly do not affect the actual counts), but it is a difficult point. Some of the young tillers which died doubtless bore eggs, but the percentage of them bearing eggs was probably the same as the percentage of the total shoots of the plot bearing eggs. In this case the death of the young tillers would affect the accuracy of neither the egg counts nor the infestation counts.

(2) Certain manures appear to have the effect of reducing infestation; but these same manures may also have the effect of increasing the number of shoots per unit area. Suppose that the fly is evenly distributed and lays absolutely at random, and that the infected shoots are consequently evenly distributed per unit area. On the plots with the most shoots per unit area the percentage infestation of the shoots will be lower than on the plots with the least shoots per unit area.

This point was tested in two ways. Firstly, the egg counts showed that the number of eggs per unit area was not the same for all plots. Secondly, the number of shoots per square yard was counted on all the Hoos Field plots when the infestation counts were made. These figures show quite definitely that the differences in the infestation of the various plots cannot be accounted for by the differences in the numbers of shoots per unit area on the plots.

EVIDENCE RELATIVE TO THE EFFECT OF MANURES ON OVIPOSITION.

On June 18th, 1923, samples of the plants were taken from plots 6_1, 7_2, 1A, 5A and 4C. For a sample of any one plot a few plants were pulled up at twenty different points on the plot. The samples were brought into the laboratory and each was examined separately for eggs. In this way both sides of every leaf in a sample were examined which would be impossible in the field. On July 2nd counts were made on these five plots and one other (plot 1AA) of the number of shoots per square yard. A yard stick was thrown on to the plot and then laid alongside that row of barley to which one of its ends (which was marked) lay nearest, and the number of shoots along a yard length were counted. This was done in five different parts of the plot and as the rows of barley are 7 ins. apart the total of the five counts gives the number of shoots per square yard. From these six counted plots samples were taken large enough to contain more shoots than would be found in a square yard of the plot in question. These were brought into the laboratory and examined for eggs as previously, except that only sufficient of each sample was examined to give the number of shoots per square yard of the plot from which the sample was taken. Thus the actual number of eggs per square yard was obtained. In the case of the first count the number of eggs per square yard was

Table I.

First Egg Count. June 18th to 26th (samples taken on June 18th).

	Plot	Infestation %	No. of shoots per sq. yd. at time of egg count. July 2nd	No. of shoots per sq. yd. at harvest	Percentage of shoots bearing eggs	No. of shoots per sq. yd. bearing eggs	No. of eggs per sq. yd.
Unmanured	6_1	14·1	225	239	20·6	47	55
Dung	7_2	4·3	463	382	15·1	70	74
$(NH_4)_2SO_4$	1A	14·2	277	258	18·9	52	59
Super. $+K_2SO_4$ $+(NH_4)_2SO_4$	5A	9·1	372	243	17	63	69
Complete minerals + Rape Cake	4C	5·9	366	315	9·5	35	42

Second Egg Count. July 2nd to 10th (samples taken on July 2nd).

Unmanured	6_1	14·1	225	239	31·5	71	88
Dung	7_2	4·3	463	382	9·5	43	43
$(NH_4)_2SO_4$	1A	14·2	277	258	18·8	52	60
$NaNO_3$	1AA	16·5	274	299	17·5	48	55
Super. $+K_2SO_4$ $+(NH_4)_2SO_4$	5A	9·1	372	243	14	52	56
Complete minerals + Rape Cake	4C	5·9	366	315	6	22	23

calculated from the number of shoots examined and the number of shoots per square yard as obtained on July 2nd; some samples included more and others less shoots than would be found in a square yard of the plot from which they were taken. Table I sets out the results of these two examinations.

The plots selected were those which showed some of the highest and some of the lowest infestations in 1922. A very unfortunate omission was made in that plot 2. O. (manured with superphosphate only) was not examined, as superphosphate is the most effective of the artificial manures in reducing infestation. All samples, to be comparable, must be taken on the same day or the samples taken later will be exposed to the chance of oviposition longer than will the earlier ones. I am indebted to Dr Imms for the advice, when I was about to start the second count, to repeat it on the plots which I had already done instead of doing an entirely fresh series; several interesting points would have been missed had I done this. All samples were taken on the same day and stood in water until the examination was finished. If the samples are done one at a time it is possible that some of the eggs will fall off the plants, and if so obviously a greater percentage will have fallen off on the samples examined last. I do not believe that the eggs do fall off under these conditions but to avoid the possibility of this error a portion of each sample was done each day until the whole count was finished; this occupied slightly over a week, and by the end of this period the plants were commencing to become mouldy. It was owing to these difficulties that only six of the Hoos Field plots could be examined.

The manuring of the plots undoubtedly has some effect upon the degree of oviposition upon the plants, and the degree of oviposition shows a distinct correlation with the final figures of infestation as is shown if the plots are arranged in order of descending magnitude as regards the percentage of shoots bearing eggs and the final infestation figures for 1923. It is equally obvious that the difference in the degree of oviposition on two plots does not explain all the difference between the final infestation of the two plots, and that on some plots a greater percentage of eggs failed to produce infection of a shoot than on other plots.

Comparing the two counts, the percentage of shoots bearing eggs is practically the same in both for plots 1A and 5A. The percentage on 7_2 is materially bigger in the first than in the second count. The plants on 7_2 grow rapidly, and at the time when the first count was made they had probably passed the stage of growth favourable for oviposition. After

this few or no eggs would be laid on this plot, and a certain number of eggs had hatched and fallen off by the time the second count was made, thus lowering the numbers. The same thing is seen in plot 4C, another rapidly growing plot. Plot 6_1 shows a much bigger percentage of shoots bearing eggs in the second count than in the first. Growth is slow on this plot and it is probable that between the first and second counts more and more shoots reached a stage favourable for oviposition. Thus plots 7_2 and 4C are normally only used by early emerging flies, whereas plots such as 6_1 are also available for later emerging flies. A certain number of eggs would, of course, fall off on all the plots between the two egg counts, but the relative numbers falling off would almost certainly be the same for all plots. On plots 1A and 5A those falling off were just about balanced by fresh ovipositions.

By comparing the final infestation of a plot with the percentage of shoots bearing eggs (in all cases the greater percentage figure of the two counts is taken here as representing more nearly the maximum oviposition upon a plot) one can obtain a figure representing the percentage of the number of shoots bearing eggs which eventually become infected. This figure is admittedly only approximately accurate. The figures are: 1AA, 94·3 per cent.; 1A, 75·1 per cent.; 6_1, 42·2 per cent.; 5A, 53·5 per cent.; 4C, 62·1 per cent.; 7_2, 25·5 per cent.; the series being arranged in order of descending magnitude of their final infestations. Thus on plot 1AA practically every shoot bearing an egg becomes infected, while on plot 7_2 only about one in every four shoots bearing eggs becomes infected. The two plots do not differ very greatly in their early growth and in their consequent liability to oviposition. They do differ greatly in their relative numbers of critical leaves (see below), and in their later growth, and consequently in the chance of a larva from a given egg infecting a shoot and attaining maturity.

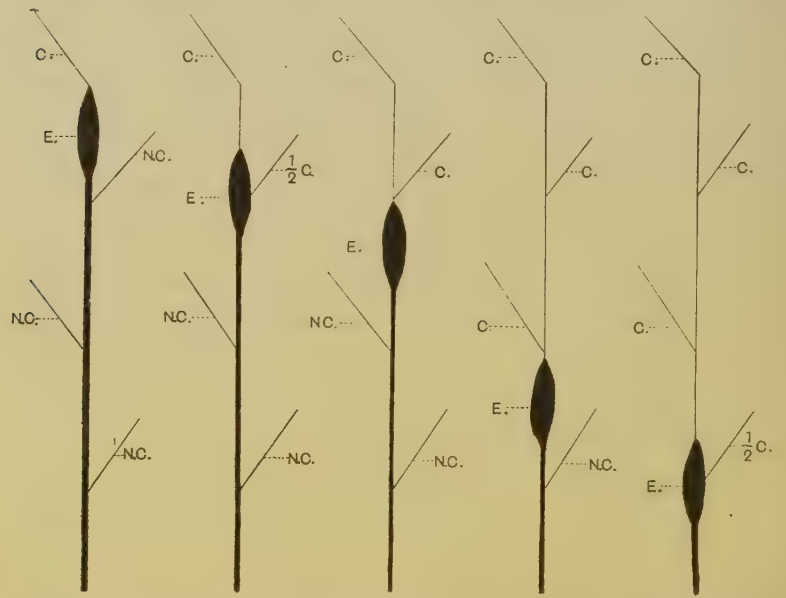
Plot 6_1 is probably the slowest growing plot on Hoos Field and one would therefore expect a greater percentage of eggs to develop than is shown in Table I[1]. I regard with considerable distrust the explanation of the discrepancy which I give below as it is almost entirely conjectural and is not rigidly based upon either observation or experiment. The ear-bearing shoots on plot 6_1 are in many cases extremely small and the size of a shoot is no index to its degree of maturity. The oviposition instinct of the fly is undoubtedly imperfect since it frequently lays eggs where, from the very beginning, and apart from the later

[1] As is shown later (p. 205) very few larvae are killed by actual rapidity of plant growth on this plot.

growth of the shoot, the larvae emerging from the eggs have no chance of developing. It is possible that on plot 6_1 the fly very frequently lays upon such unsuitable plants. It must be pointed out that what occurs on plot 7_2 is in the main entirely different. Here the shoots selected for oviposition are usually suitable at the time when oviposition occurs, but during the incubation period of the egg the shoot grows so rapidly that by the time the larva emerges from the egg the plant is unsuitable[1].

EVIDENCE RELATIVE TO THE EFFECT OF MANURES UPON THE LARVAE.

The following theory as to the action of the manures upon the larvae was formulated at the time when the egg counts were done in 1923. It will best be understood by reference to the diagrammatic Text-fig. 2. The



Text-fig. 2. Diagram of barley shoots showing, from left to right: 1, 1½, 2, 3 and 3½ critical leaves. *C* = critical leaf; ½*C* = half critical leaf; *E* = ear; *N.C.* = non-critical leaf.

whole question turns upon the position of the ear with regard to the leaves; if an egg is laid upon a leaf lamina whose base comes off from the shoot below the base of the ear, the first instar larva will enter the shoot below the ear. If the egg is laid upon a leaf arising very low down upon a shoot

[1] 7_2 also differs from 6_1 as regards critical leaves (see below).

the young larva will even reach the plant below the ear-bearing internode. It is an invariable rule that when a larva enters the shoot it moves downwards, and therefore if it enters below the ear it can never get on to the ear to feed. All leaves above the ear of a shoot may therefore be termed the "critical leaves," *i.e.* a larva from an egg laid upon any of these leaves will have a chance of infecting the shoot. A leaf arising from the shoot half way up the level of an ear is considered as half a critical leaf because the larva from the egg on such a leaf would enter the plant upon the ear though not upon its tip. The ovipositing instinct of the fly is not perfect and eggs are frequently laid upon leaves arising from the shoot below the ear. It was thought that if a larva failed to get on to the ear it would die; this is not strictly accurate as the 1923 examinations have shown that a larva can feed for a certain length of time upon the ear-bearing internode alone, and in certain very rare cases it will even attain maturity under these conditions: usually it dies in the second instar or early in the third instar. These cases will be considered later but they are exceptional and do not affect the theory. Comparing plots 7_2 and 6_1:

It was supposed that owing to the greater growth on plot 7_2 there would be on the average, fewer critical leaves per shoot upon this plot than upon 6_1. Consequently a greater proportion of the eggs upon 7_2 would be laid upon unsuitably placed leaves than would be the case on plot 6_1. Further, suppose an egg to be laid upon a leaf arising from the shoot just below the tip of the ear (*i.e.* a "$\frac{1}{2}$-critical leaf") its chance of infecting the shoot would be greater in plot 6_1 than in plot 7_2 because growth is more rapid in the latter. The incubation period of the egg is about 8 days. During this time the ear in the shoot on plot 7_2 has a very good chance of growing up above the leaf upon which the egg is laid; the ear of the shoot upon plot 6_1 has a very much less chance of doing so.

There is one other way in which the manures occasionally react upon the larva and although very rare (according to the 1923 observations) it deserves some mention. An egg may be laid upon a leaf which is entirely suitable as regards position and the young larva may enter the shoot on, or above, the apex of the ear and yet may fail to develop because the ear, though still within its sheathing leaf, is too mature and tough for the young larva. Practically no damage is done to the plant in these cases.

Three bodies of evidence have been collected in support of the "critical leaf" theory, one experimental, which I shall therefore present first, and two observational.

The whole "critical leaf" theory could have been rigidly tested under controlled experimental conditions if the oviposition experiments had been successful. It was suggested to me by Dr A. D. Imms that it would be well worth while to try to test the point in the open although strict control would in this case be impossible. Accordingly on July 9th, 12 shoots bearing eggs were selected along one side of plot 6_1: they were selected as having eggs upon only one of the leaves. Each shoot was marked and a note made of the number of critical leaves upon each shoot and the number of the leaf (counting from the apex downwards) upon which the eggs were laid. A prediction was then made as to whether the shoot would become infected or not. At the time it was not known that a larva which failed to get on to the ear of a shoot could still damage the internode; if this had been known the predictions would have been somewhat modified.

Table II.

Shoot No.	Eggs (July 9th)	Prediction	Final observation (Aug. 22nd)
1	3½ critical leaves. Egg on leaf no. 4	May or may not be infected	Not infected
2	2½ critical leaves. Egg on leaf no. 4	Will not be infected	,,
3	2½ critical leaves. Egg on leaf no. 4	,,	Infected but only the internode damaged. Ear untouched
4	2½ critical leaves. Egg on leaf no. 4	,,	Infected but only the lower half of the internode damaged
5	4 critical leaves (no sign of ear). Egg on leaf no. 3	Will be infected	Infected. Damage from apex of ear downwards. Shoot remained very small, not much distorted but ear almost completely destroyed
6	2½ critical leaves. 2 eggs on leaf no. 4 (one hatched)	Will not be infected	Infected. Very shallow groove down middle of internode. Internode not in the least distorted and over 9 ins. long. Larva certainly did not mature
7	4 critical leaves. Egg on leaf no. 3 (no sign of ear, apical leaf only just appearing)	Will be infected	Infected. Shoot remained small. Distortion slight. Ear almost completely destroyed
8	4 critical leaves. Egg on leaf no. 3 (no sign of ear)	,,	Infected. Slight damage on internode only
9	3½ critical leaves. Egg on leaf no. 3	,,	Ear had failed to come out of sheathing leaves but no certain sign of *Chlorops* damage could be detected
10	3½ critical leaves. 2 eggs on leaf no. 3	,,	Definitely not infected
11	3½ critical leaves. Egg on leaf no. 4	May or may not be infected	Not infected
12	3½ critical leaves. Egg on leaf no. 3	Will be infected	Infected. Distortion not great but ear almost completely destroyed

Nos. 1, 2, 5, 7, 11 and 12 follow the prediction. Nos. 3, 4 and 6 also follow the prediction in the sense that when this was made the type of infection where only the internode is damaged was not known, and the prediction does not therefore take into account this type of damage. No. 8 follows the prediction but I cannot understand why, in this case, the damage is to the internode only. Either there was quite abnormally rapid growth in this shoot after the egg was laid or else the egg observed did not hatch and an egg laid at a later date is responsible for the damage. This is the great objection to carrying out this experiment in the open—one cannot be sure that eggs are not laid upon the shoots after the observations are made. One can be fairly sure that infection has not taken place prior to the observations because in the young shoots the very first sign of distortion can be detected by a careful examination. No. 9 is a doubtful case. No. 10 should have been infected and was not; this is the only instance where the result is directly opposed to the prediction. This was the biggest shoot and had the biggest ear of the whole twelve and it may be that this is one of the cases previously referred to where the ear, though in the right position for infection, is too mature and tough for the larva. Unfortunately birds had been feeding on this ear so that it could not be carefully examined for minute signs of attack at the apex of the ear. I think therefore that this experiment offers strong support to the critical leaf theory; it does not, and could not be expected to afford definite proof since the experiment could not be carried on under critical conditions.

Assuming the critical leaf theory to be correct, if one could at the time of oviposition arrange the plots on Hoos Field in a series according to the relative numbers of critical leaves per shoot on the different plots, the order of the series should correspond with the order of the final infestations. This test cannot be carried out really accurately because to be of any value all plots considered must be examined, as regards their relative number of critical leaves, upon the same day, and an examination of several thousand shoots on each plot would be necessary for real accuracy. An eye estimate of a single observer is valueless being liable to very grave errors, but the error is considerably less if four observers estimate the critical leaf value of a plot and the mean of the four observations is taken, and it was suggested by Dr Imms that observations might be made in this form on a few plots which were expected to give considerable differences in the final infestation figures. I am greatly indebted to Dr Imms, Mr Garner and Mr Eden for having enabled me to make the observations in this form. All four observers examined the same plot together; when the examination was finished

figures were agreed upon which represented the mean of the four opinions. The following two examples may be given:

Plot 4C.	0 to 1	critical leaves on	90	%	of the	shoots
	1½ or more	„ „	10	„	„	
Plot 1AA.	0 to 1½	„ „	0	„	„	
	2	„ „	15	„	„	
	2½ or more	„ „	85	„	„	

Plot 1AA is, from the above figures, obviously more liable to infection than plot 4C. The estimates of the four observers were usually remarkably close. Plots 1AA, 6_1, 1A, 5A, 7_2 and 4C were examined in this way and placed in the order given as regards the number of critical leaves per shoot, 1AA having the most and 4C the least. The correspondence of this order with the order of the same plots as regards their final infestation figures is truly remarkable when it is remembered that their order as regards relative numbers of critical leaves is based upon estimate only, which cannot be expected to detect small differences with accuracy.

The last body of evidence relative to the effect of manures upon the larvae and supporting the critical leaf theory is drawn from a very careful examination of infected shoots on plots 1AA, 1A, 6_1, 5A, 4C, 2. O., and 7_2. When the infestation counts were done on these plots at the end of July 1923, the infected shoots from each sub-count of 100 shoots (see above, p. 193) were brought into the laboratory and carefully examined, there being thus ten samples from each plot. It is impossible to give this evidence in full.

In many instances (36 altogether) a young larva was found dead upon the ear-bearing internode and in all these cases the internode only had been damaged. It was absolutely certain that in these cases the first instar larva had entered the shoot below the ear and had therefore commenced its feeding upon the internode where it had not been able to obtain sufficient nourishment, the internode being too tough, and it had consequently died. The damage done to the internode was usually very slight, the food groove being in some cases less than ½ in. in length. Even in these cases the ear failed to emerge fully from the sheathing leaf and the distortion of the plant, though slight, was sufficient to mark it out in the field as having been attacked. The degree of distortion of the plant varies to some extent according to the amount of damage done to the internode and this depends mainly upon the degree of hardness (*i.e.* maturity) of the internode when the young larva commences feeding. If the internode is fairly young and soft the larva may contrive to feed for some time and may even get as far as the beginning of the third instar

before dying from insufficient food. Two cases have been seen in which a larva attained maturity and pupated although only the internode was attacked. Besides the 36 cases in which the dead larvae were actually found there were 69 cases of slight damage to the ear-bearing internode where the larvae were not found but were, with I think perfect safety, assumed to be dead. In examining large numbers of infested shoots (about 700 were examined on the seven plots mentioned above) it is impossible to spend time searching for a minute dead first or second instar larva if it does not happen to have died in the food groove; the dead larvae are moreover liable to fall out in opening the shoot for examination.

All the larvae causing the above type of damage must have failed to produce the typical damage to the ear for one of two reasons. Either the eggs from which the larvae emerged were laid upon a leaf arising from the shoot below the base of the ear, or else, although originally laid upon a suitable leaf, the rapid growth of the ear during the period of incubation of the egg had carried it above the leaf in question by the time the larva emerged. If this is so more larvae should be killed in this way upon rapidly growing plots than upon others and this is undoubtedly the case as the following figures show. The plots are arranged in order of descending magnitude of infestation:

	1AA	1A	6_1	5A	2. O.	4C	7_2
Larvae killed by rapidity of plant growth	15·25 %	4·2 %	9·2 %	18·7 %	35·8 %	23·7 %	25·6 %

I cannot explain the high figure on plot 1AA which is manured with $(NH_4)_2SO_4$. Excessive quantities of nitrogenous manures retard the maturity and growth of the ear and ear-bearing internode (on Rothamsted soils) and one would therefore expect few larvae to be killed by the plant growth on this plot. The expected effect of nitrogenous manures is shown in plot 1A which is manured with $NaNO_3$. The figure for plot 2. O. (manured with superphosphate) is extremely interesting, being the highest of all. It has already been mentioned that superphosphate is the most striking of the artificial manures in reducing gout fly infestation. One of the most firmly established facts regarding the effect of superphosphate upon the plant is that it hastens maturity. This is precisely what is indicated by the large percentage of larvae which are killed by the growth of plants manured with superphosphate. The number of larvae killed on the other four plots fits in very well with the relative rates of growth on these plots as far as this is known.

A particular type of damage must be mentioned here. One occasionally comes across shoots bearing an ear upon a long uninjured internode

which is, however, usually twisted and bent (sometimes extraordinarily so) and arises from a basal region which looks exactly like a typical winter form of attack except for the fact that the stem and ear arise from it. For a long time I took these to be shoots which had been attacked and had subsequently "recovered," and I thought that they formed an exception to the rule that once growth has been stopped by larval attack it will not recommence. I think now that this type of deformation is due to an egg being laid upon one of the lowest leaves of a very young shoot, *i.e.* upon a leaf far too low down to be a critical leaf. In a few cases the larva might get into the base of the shoot and feed for a short time before dying on account of its unsuitable position. It may be supposed to feed long enough to inhibit the growth of the young leaves and the lower internodes of the stem which are just forming. For some reason which I cannot explain, its inhibiting influence is not sufficiently powerful to stop growth of the ear and ear-bearing internode although its influence is shown in the deformed wavy growth of the internode. Whether this view is correct I cannot say as I have never been able to find the dead larva in this type of shoot (which is very rare), and the above is not brought forward as evidence in support of the critical leaf theory. It is merely an abnormality which seems to be explained by the theory. Taking into consideration the whole of the evidence given above, I think that the following statements may be regarded as definitely established.

Manurial treatment affects the infestation by gout fly of summer barley by:

(1) Altering the percentage of shoots which, at the time of oviposition, are in a stage of growth favourable for oviposition.

(2) Altering the rate of early growth of stem and ear and consequently altering the number of leaf laminae which, at the time of oviposition, will arise from a shoot above the level of the apex of the ear: only eggs laid upon these leaves can give rise to the typical damage.

(3) Altering the rate of growth of the ear-bearing internode during the period of incubation of the egg, and consequently altering the number of ears which, during the incubation of the egg, will grow up above the leaf bearing the egg and so escape infection.

(4) Altering the degree of maturity (and consequently the toughness) of the ear and ear-bearing internode. This may usually be regarded as forming part of effect No. 3. As a separate effect it is very rare and may be disregarded.

Thus the entire effect of the manures upon infestation is due to their

effect upon the plant growth and is not a direct effect upon the larval nutrition. The determination of this fact is of considerable importance in several ways.

(1) If the manures act directly upon the nutrition of the larva they should act in the same way in all years and in all parts of the country. As, however, their action is upon the growth of the plant their effect may be expected to vary under different climatic conditions and upon different types of soil. This is in fact the case.

(2) If the manures act directly upon the larva they might be expected to have similar effects on other Acalyptrate larvae (and quite possibly on the majority of insect larvae) feeding on and living within the shoots or leaves of cereals. Since they act only by their action on plant growth this generalisation cannot be made. Each pest must be considered separately with regard to its interrelation with the growth of the plant: only then can be estimated the possible effect of a particular manure whose effect upon plant growth is known.

(3) If the manures had been found to be acting upon the larva (*e.g.* by altering the constitution of the cell sap of the plant in such a way as to lower its feeding value for the larva or possibly making it even injurious to the larva) this would have opened up the possibility of dressing the soil with substances other than those usually used as manures. It might have been possible to discover a substance which could be absorbed in small quantities without injury to the plant, but which would, directly or indirectly, alter the constitution of the cell sap in such a way as to render it harmful to the larva. Injury to the plant would, of course, only be prevented if the larva was killed at an extremely early stage, but the repeated use of such a substance would end in the almost complete eradication of the gout fly. I am indebted to Dr Davidson for permission to refer to some recent and as yet unpublished work of his upon *Aphis rumicis* which proves that, for this insect at all events, substances added to the soil in which the host plant is growing can affect the infestation numbers. The efficiency of different manures in reducing infestation by gout fly being entirely bound up with their efficiency in promoting early plant growth, the utility of searching for a soil dressing such as that mentioned above becomes negligible.

THE EFFECT OF LATE DRESSINGS WITH NITROGENOUS MANURE.

A dressing of nitrogenous manure when the plants show signs of attack has several times been recommended as a remedial measure for plants attacked by the summer generation of *Chlorops taeniopus*. I have

no hesitation in saying that when once a larva is established in a shoot no remedial measures are possible. Nothing will cause growth to recommence when once it has been stopped by the larva; even the removal of the larva (unless at the very beginning of its life) will have no effect in this way. It is therefore useless to wait until signs of attack are apparent before applying a manure. The following experiment was set up to determine whether late dressings of nitrogenous manure would have any effect if applied immediately before oviposition commenced or if applied when oviposition was mainly finished. A plot of barley was marked off on Hoos Field; the plot was 18 ft. long and 10 rows of barley ran through its length. The plot was divided transversely into three and longitudinally into two, thus dividing it into six plots each 2 yards long and containing five rows of barley. Plot XP was given 31·7 gms. (= $1\frac{1}{2}$ cwt. per acre) of $NaNO_3$ on May 29th and plot ZP was given the same dressing of $(NH_4)_2SO_4$; YP was given no nitrate. Plot XQ was given 31·7 gms. of $NaNO_3$ on June 14th and plot ZQ was given the same dressing of $(NH_4)_2SO_4$; YQ was unmanured. The plot was harvested on August 23rd and each row of each plot examined separately in the laboratory. The mean infestation of the five rows for any plot was taken as the infestation figure for the plot. The infestation percentages are as follows:

YP, 6·66 %; ZP, 6·13 %; XP, 2·31 %; YQ, 10 %; ZQ, 7·3 %; XQ, 6·36 %

XP is the only plot upon which marked reduction of infestation has been obtained, and I am indebted to Mr R. A. Fisher, who has examined these figures, for the statement that the reduction is quite significant. The difference of infestation between YP and YQ, which were both unmanured and should therefore have given the same figure of infestation, though rather large, is not significant.

Thus the application of $NaNO_3$ to the plant at the commencement of the period of oviposition appears to have helped on the growth of the ear and stem and so reduced infestation. The $NaNO_3$ applied on June 14th was too late to produce any effect on growth before the eggs had hatched and the larvae penetrated into the plant. The $(NH_4)_2SO_4$ dressing had no effect either as an early or a late dressing. Mr Fisher suggests that this may be due to delayed action on the part of this manure owing to the time taken for its nitrification. If so an earlier application of the $(NH_4)_2SO_4$ would have caused a reduction in infestation.

In spite of this experimental result late dressings of nitrogenous manures cannot be recommended as preventative measures against

gout fly attack. The area upon which this experiment was carried out is a part of Hoos Field, outside the experimental plots, which carries barley every year but which has for many years received no manure. The land is therefore impoverished. In ordinary farm practice barley would not be grown upon such poor land and to give such a dressing as was used in this experiment might result in giving the plants an overdose of nitrogenous manures. As is shown on some of the plots on Hoos Field, repeated heavy dressings of nitrogenous manures have no beneficial effect in reducing infestation and may even have an adverse effect. I am indebted to Dr W. E. Brenchley for some interesting notes concerning the effect upon the growth of barley of varying quantities of nitrogenous manures. These notes show that while a small quantity of nitrogenous manures ($NaNO_3$ was used) may accelerate the growth of the ears, an excessive dressing has a very marked effect in retarding such growth. In districts where gout fly attack is bad, nitrogenous manures should therefore be used with extreme care; any retarding action which they may tend to show may be overcome by a liberal application of superphosphate.

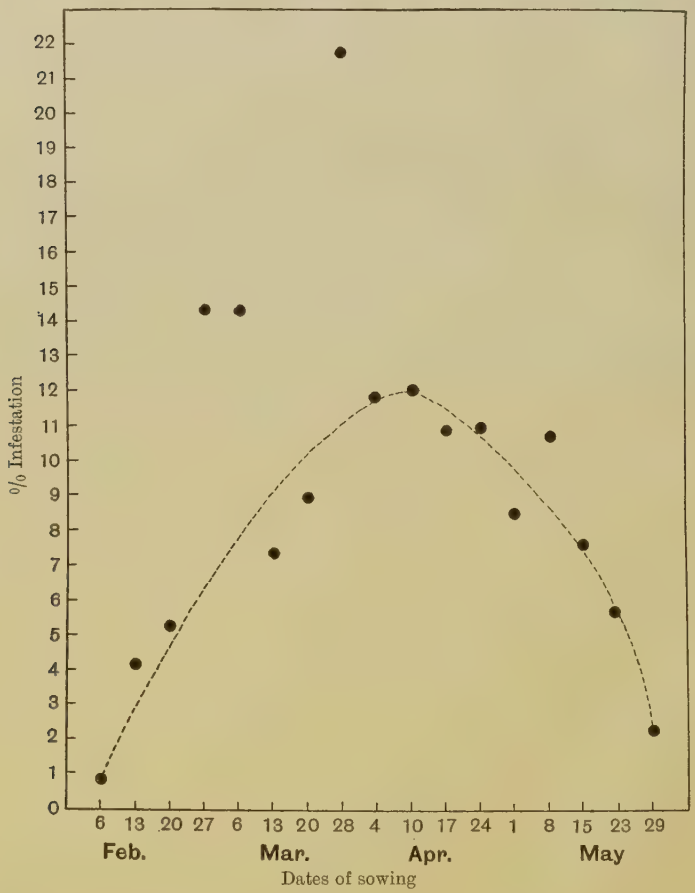
RELATION OF TIME OF SOWING TO INFESTATION.

The importance of early plant growth and maturity in reducing the infestation has already been emphasised, and one might safely assume from this that the earlier spring barley is sown the less it will suffer from gout fly attack provided the soil conditions are favourable for the early sowing. The comparative immunity of early sown spring barley from attack by gout fly has, indeed, been noted by many observers.

In 1923 an attempt was made to investigate experimentally the influence of times of sowing. A plot was marked off on a part of Hoos Field (2 yards by 25 yards) and six rows of barley were sown (except the first two sowings which were five rows each) every week from Feb. 6th until May 29th. The barley was sown regardless of soil conditions which were in many cases, particularly for the early sowings, extremely bad. The soil on Hoos Field is extremely unfavourable for the success of such an experiment as this; to obtain good soil conditions in the heavy soil of this field the time of working the soil is of the greatest importance, and has to be chosen with great care. Obviously no attention is paid to this when sowings of barley are made on fixed dates. The actual percentages of infection for the various dates of sowing cannot therefore be considered as really accurate. They do, however, show definitely the relation of infestation to time of sowing. Far better figures would

probably be obtained by repeating the experiment on a lighter soil which would give a greater degree of uniformity of soil conditions throughout the experiment.

The plot was harvested on August 22nd, the plants being uprooted



Text-fig. 3. Curve showing the correlation of times of sowing of spring barley and its infestation by gout fly.

instead of being cut so that all shoots were obtained. Each row was examined separately in the laboratory, the mean infestation figure for the six rows planted at each time of sowing being thus obtained. The results are shown graphically in Fig. 3. Fourteen of the seventeen means

fall fairly close to the smooth curve shown on the graph. The infestations are very low for the early sowing, attain a maximum for the rows sown on April 10th and then fall off, slowly at first but more rapidly for rows sown after May 8th. This is precisely the type of curve which is expected. The early sown plants are not heavily infected because they are too well grown at the time of oviposition. The very late sown plants (those sown after May 8th) did not germinate until the end of May and beginning of June. By the time they had made sufficient growth to be suitable for oviposition there were very few *Chlorops* about. Such late sowing as this is, of course, impossible in practice as the barley would not ripen except in an extremely exceptional year.

The infestations of the rows sown on Feb. 27th and March 6th do not fall on the smooth curve and are exceptionally high. The high infestation is probably due to a caking of the soil surface which was noticed at the beginning of April to be particularly bad on these twelve rows. This, of course, hindered plant growth very seriously. The cultural conditions were bad for all the first six sowings but the two mentioned above were probably those which were most seriously affected.

Rows 41–46 (sown March 28th) also show an abnormally high figure of infestation. I can find nothing to account for the exceptionally poor growth upon these six rows but they were undoubtedly very poor. Rows 41–46 gave, at harvest, only 46 barley shoots compared with 112 on rows 35–40 and 196 on rows 47–52. Rows 41–46 were therefore suffering from adverse cultural conditions in some way and hence the high figure of infestation.

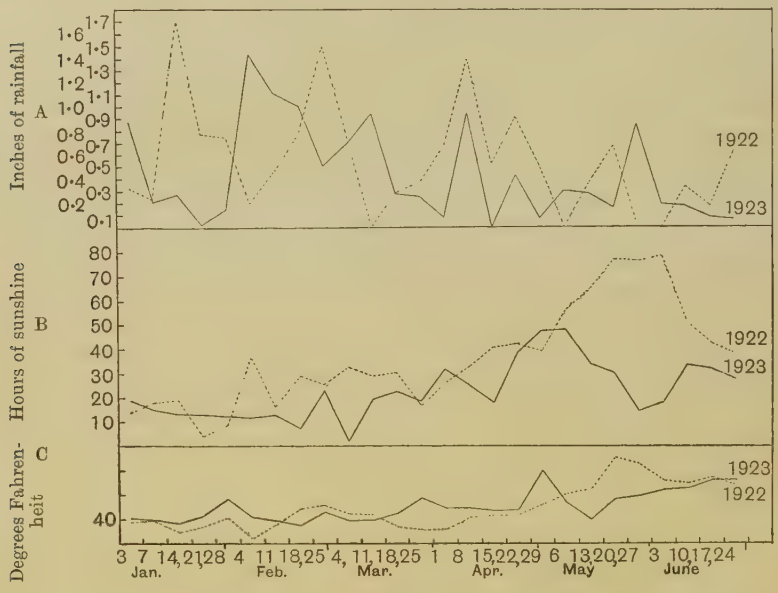
The experiment shows therefore that time of sowing of barley has a marked effect upon its infestation by gout fly but the experiment needs repeating upon a more favourable type of soil and it is hoped that this will be carried out in 1924.

COMPARISON OF 1921–22 AND 1922–23 IN REGARD TO GOUT FLY.

Some of the most useful information that can be obtained with regard to any plant pest is that which enables one to correlate the abundance of the pest in any year with the meteorological conditions of that year and the preceding year. The ability to predict the probable infestation in any year would give a sound basis for recommending what preventative or remedial measures may profitably be undertaken. Such correlation obviously cannot be made upon only two years' observations, but a few probable correlations may be pointed out. The following observations refer almost exclusively to the summer generation which is

by far the more important of the two as regards the damage done, and regarding which I have a far greater mass of observations than I have concerning the winter generation.

It is obvious in the first place that good or bad conditions for plant growth during April and May will cause respectively less or greater infestation by gout fly. The conditions for plant growth during this period were probably slightly better in 1922 than in 1923, yet comparing



Text-fig. 4. Chart showing weather conditions for the first six months of 1922 and 1923. A and B, the weekly totals of daily readings. C, the weekly means of daily maximum and minimum air temperatures. (The 1923 graphs are shown by a firm ine, those for 1922 by a dotted line.)

the infestation upon the Hoos Field plots for the two years one finds that in the majority of cases the infestation was higher in 1922 than in 1923. The reason is almost certainly as follows. In 1923 the flies emerged slightly later in spring than they did in 1922. In 1922 the weather at the end of May and beginning of June (*i.e.* the period of oviposition) was bright and sunny and the temperature was high. Oviposition was consequently stimulated and eggs were laid rapidly and prolifically. In 1923 the corresponding period was very dull and the temperature was con-

siderably lower than in 1922; from oviposition experiments done at this time it is certain that under these conditions the flies lay very few eggs. Plant growth would undoubtedly be slowed down to some extent by the cold dull weather but would probably not be slowed down very seriously, and the plants would in many cases grow beyond the stage favourable for oviposition before the flies had laid upon them. It was probably because of this that in 1923 a considerable proportion of the flies utilised couch grass as the summer host instead of barley; in 1922 practically the whole of the summer generation was upon barley.

The flies emerged a few days later in the spring in 1923 than in 1922; if adverse conditions during the previous winter can delay the emergence of the flies for an appreciable time (*i.e.* a fortnight or more) this would almost certainly result in a very much smaller infestation of the spring barley of the following year because, presuming the spring conditions to be favourable, the plants would make good growth before oviposition commenced. Whether a hard winter would affect the winter larva in such a way as to retard the emergence of the flies I am unable to say as the winters 1921–22 and 1922–23 were both mild ones.

Comparing the summer attack on barley in 1922 and 1923 a very distinct difference in the type of damage was noticeable. In 1922 a large proportion of the attacked shoots showed the winter type of damage, and many of the others were of non-typical summer type. In 1923 the majority of the attacked shoots were of the typical form. This means that, owing to the delayed oviposition of the flies in 1923 on account of the dull weather, the plants were on the average more advanced in growth when oviposition did occur than they were in 1922. This is further shown by the relatively large number of cases in 1923 where the larva only damaged the internode.

As already mentioned, very little damage caused by the summer generation has been seen on wheat. In 1922 the wheat on New Zealand Field was damaged to the extent of possibly 3 or 4 per cent. (counts were not made) and this is the only case where the damage has been found on wheat. The reason is that wheat is an autumn sown crop (in this district) and is consequently in an advanced stage of growth in early June when oviposition occurs. The same applies to winter barley. It must not be inferred, because wheat and winter barley are not attacked by the summer generation, that the plants are inherently less susceptible to attack by gout fly than is spring barley; they may be so but their immunity is more probably due to their advanced stage of growth at the time of oviposition. In the case of New Zealand Field mentioned

above the wheat had made extremely poor growth and was the most backward wheat on the whole farm.

It has been suggested by many writers (see (3) for full details) that an autumn generation of *Chlorops* may occur. Nowicki (3) considers that this may occur in certain conditions, *i.e.* when a favourable summer has led to the emergence of flies very early in autumn. As already mentioned I did not succeed in rearing an autumn generation from flies emerging early in August 1922. It cannot, of course, be deduced from this that an autumn generation never occurs, but if it does occur I imagine that even in the most favourable conditions (*e.g.* the summer of 1921) it would be only a partial generation due to a few exceptionally early emerging flies.

The probable influence of weather may then be summed up as follows. A cold winter may retard emergence of the flies in the following spring and so allow the barley to reach an advanced stage of growth before oviposition occurs. Good growing weather during April and May will reduce infestation for the same reason. Dull cold weather during the end of May and beginning of June is more disadvantageous to the flies than to the plants and will reduce infestation. A hot summer may favour the development of a partial intermediate (autumn) generation.

PARASITISATION OF *CHLOROPS TAENIOPUS.*

Up to the present observations have been made upon four Hymenopterous parasites of *Chlorops taeniopus*, and I hope shortly to deal with these in some detail in a separate paper. In this place I shall only mention briefly the effect upon the parasitisation of *Chlorops* of different times of sowing of the host plant and different manurial treatments. Upon the latter point I have extensive data drawn from the 1923 examination of plots 1AA, 1A, 6_1, 5A, 4C, 2.O. and 7_2.

Of the four parasites two—*Coelenius niger* (a Braconid) and *Pteromalus micans* (a Chalcid)—are endoparasites of the *Chlorops* larva while two others (not yet identified) are ectoparasites. The two former have been found in both winter and summer generations but the two latter have so far been found only in the summer generation. The degree of parasitisation of the *Chlorops* larvae taken from each of these plots mentioned above has been obtained for each of the four parasites and the results are extremely interesting. Here it will be sufficient to mention the total parasitisation only. This is as follows:

1AA, 18%; 1A, 25·19%; 6_1, 43·93%; 5A, 14·47%; 4C, 9·76%; 2.O., 7·7%; 7_2, 10·8%

The details of the causes of this variation in parasitisation cannot be given here. Briefly the variation is caused by the fact that plots such as 4C, 2. O. and 7_2 are utilised mainly for the earliest oviposition. The imagines of the parasites emerge from their winter generation later than the *Chlorops* imagines and the endoparasites only attack the very young stages of the *Chlorops* larvae. The very early *Chlorops* larvae therefore escape parasitisation by the endoparasites; there was not a single endoparasite in the 90 larvae examined from plot 2. O. and plot 7_2. The ectoparasites, as might be expected, attack a later and more mature stage of the *Chlorops* larva and the early *Chlorops* larvae do not therefore escape parasitisation by these.

The effect of time of sowing upon parasitisation is only known by analogy with the above results. The infected shoots on the experimental plot set up to determine the effect of different times of sowing upon infestation of the plants by gout fly, could not be examined for parasitisation as it was necessary for the purpose of the experiment to leave the plants growing until harvest time. It is fairly certain that the larvae in very early sown plants would be little parasitised and that parasitisation would increase in the later sowing.

The same type of result is obtained for the winter generation of *Chlorops* except that very late *Chlorops* larvae which are sometimes found (see above, p. 190) are not parasitised at all because the imagines of the two endoparasites which attack the winter generation do not live on after cold weather has set in. The *Chlorops* imagines themselves are much less susceptible to cold. The parasitisation of the winter generation of 1922–23 was in general very high, averaging about 40 per cent.

These results demonstrate that all measures taken for the promotion of early plant growth will have the unfortunate effect of reducing the parasitisation of the summer generation. One would greatly have preferred finding the reverse effect. It is difficult to estimate the amount of importance which should be attached to this effect upon the parasites. Probably it is not so serious as the above figures suggest. If by the promotion of rapid early growth spring barley crops are rendered unsuitable as host plants for *Chlorops*, couch grass will be utilised instead. This happened to quite a considerable extent in 1923 (owing rather to the delayed oviposition of the flies than to good early growth of the plants). I did not examine infected couch grass shoots in the summer of 1923, but it is probably safe to assume that the larvae in such shoots would be parasitised to a normal extent. The couch grass would therefore tend to stabilise the balance of *Chlorops* with its parasites. It might

even tend to increase the percentage of parasitisation because a large part of the attacked couch grass would be on root fields which are constantly cultivated during the early stages of growth of the roots; many of the early larvae would be destroyed owing to the death of their hosts due to this cultivation. The later larvae would not be destroyed to quite the same extent and it is the later larvae which are most heavily parasitised. This is rather speculative but I hope to obtain some observations on these points in 1924[1].

PREVENTIVE MEASURES.

General. The eradication of couch grass would have a very great effect in reducing the total numbers of gout flies. Complete eradication of couch grass is, of course, impossible, but it can be kept under control on arable fields and particularly on root fields where it is, apparently, most liable to attack.

Summer generation. This is the generation which does the greatest damage. The most effective measure to guard against attack is very early sowing of the spring barley. In heavy cold soil sowing cannot usually be done very early but depends, far more than is the case with light warm soils, upon weather conditions. Very early sowing on heavy cold land would probably cause far more damage to the crop by poor cultural conditions than would have been caused by the gout fly had the crop been sown later. Here, then, very early (*i.e.* February or early March) sowing is impossible though the sowing should be made at the very earliest date permissible with regard to soil conditions. It is upon soils of this type that superphosphate is particularly useful as a manure, as it stimulates rapid growth and early maturity of the ear and ear-bearing internode.

Any manure which will stimulate the early growth of the plant and tend to force up the ear and ear-bearing internode will have a beneficial effect in reducing infestations. It is by no means certain that artificial manures always react in the same way upon plant growth; their action may vary with variations of soil type and climatic conditions. Superphosphate seems to be remarkably constant in its action in promoting early maturity and the use of this manure is almost certain to have beneficial results in reducing gout fly infestation. Farmyard manure

[1] Some data concerning the parasitisation of the larvae of the summer generation of *Chlorops* on couch grass had been overlooked when the above was written. Three separate counts were done from July 18th to July 21st, 1923; in all 191 *Chlorops* larvae were examined, and the figures tend to confirm the speculations given above. The *Chlorops* larvae were parasitised to the extent of 41 %—the endoparasites being markedly predominant.

seems also to be uniformly beneficial. The necessity for caution in applying nitrogenous manures has already been indicated. With regard to other artificial manures no general recommendation can be made. Thus in 1922, K_2SO_4 had a very marked effect in reducing infestation, but this effect was not repeated in 1923. These points will be found in greater detail in the analysis of the manurial counts by "Mathetes" (5).

Good cultural conditions of the soil are naturally of great importance as they have a great effect upon plant growth. It is because of the poorer cultural conditions that the headlands of a field of barley are often more heavily attacked by gout fly than the rest of the field.

Arthur (*Gardener's Chronicle*, 1844, p. 365) recommends the application to the land of noxious perfumes "such as soot, guano, or sand immersed in gas water" to prevent the fly alighting on the fields. I can offer no opinion as to whether this would be efficacious or not and the recommendation does not appear to be based upon any definite observation or experiment.

Winter generation. The damage done to winter barley and winter wheat crops is, so far as I have seen, quite negligible. This is not always the case; Jablonowski gives the following figures (quoted in (2)) for the winter 1909–1910. Wheat, 34·18 per cent. attacked; Rye[1], 60·10 per cent. attacked. Late sowing of autumn crops will minimise the attack, but the sowing must not be so late as to retard the growth in the following spring or the plants may be attacked by the summer generation. Fulmek (2) gives a very clear account of the points to be considered in connection with the winter generation. The actual damage done by the winter generation is probably not great as the shoots killed will be replaced by fresh tillers which will develop sufficiently early to bear good grain. Nevertheless if the winter attack is disregarded and the seed sown early in autumn a plentiful supply of winter hosts is being provided and the spring barley of the next year is liable to suffer badly. It is therefore advisable to sow winter wheat and barley as late in the autumn as can be done with safety, having regard to growth conditions.

The danger of winter hosts other than couch grass and winter cereals has already been indicated and means of prevention given (pp. 189–190).

SUMMARY.

Chlorops taeniopus passes through two generations per year. The winter generation is mainly upon couch grass but also occasionally upon

[1] Rye is not grown in the Harpenden district and I have no observations concerning the attack of gout fly upon rye. It does occur in this country.

winter wheat or barley or upon self-sown wheat or barley. The summer generation is mainly upon spring barley but in seasons unfavourable to the fly couch grass may be utilised. Very rarely wheat is a summer host plant. The life-history is described in detail.

The type of distortion caused to the host plant depends on the stage of growth of the plant when attacked and the degree of distortion of the plant depends upon the rate of growth at the time of attack.

The relation of the fly to the different kinds of host plants is described particularly as regards the winter generation, and is shown to vary with such factors as date of emergence of the flies, weather conditions during the oviposition period and amount of growth of the different kinds of host plants.

In dull and cool weather the flies will lay few eggs but are stimulated to rapid egg laying by bright and sunny weather. A single fly may lay about 150 eggs. More than one act of coitus is necessary to fertilise all the eggs which a ♀ is capable of laying. The length of life of the imagines is probably about a fortnight for flies emerging in spring but may be over two months for the autumn emerging flies.

Certain manures (particularly superphosphate) are shown to have a marked beneficial effect in reducing the infestation of summer barley by gout fly. The effect of the manures is shown to be due entirely to their stimulating effect upon the maturing of the ear and the growth of the ear-bearing internode.

It is shown that while small dressings of nitrogenous manures may reduce infestation, large dressings will not reduce it and may have a tendency to retard growth of the ear and so increase infestation.

Early sowing of spring barley is shown to be efficacious in preventing infestation by gout fly.

Preventative measures suggested are early sowing of spring barley, good cultural conditions of the soil, and manuring (*e.g.* with superphosphate or farmyard manure) to stimulate early growth.

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(4) Ormerod, E. A. (1890). Manual of Injurious Insects and Methods of Prevention (2nd ed. London, pp. 75–79).

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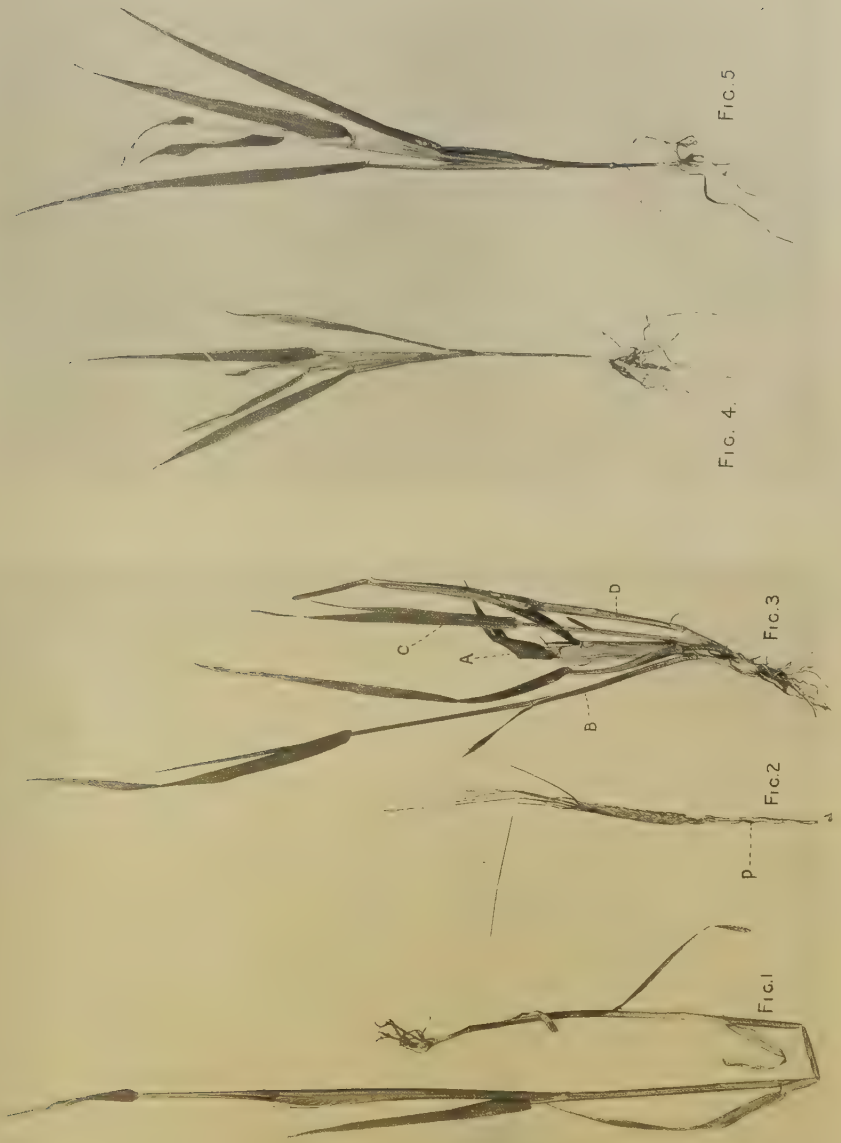
FIG 1

FIG. 2

FIG 3.

FREW.—ON *CHLOROPS TAENIOPUS* MEIG. (THE GOUT FLY OF BARLEY). (pp. 175—219.)



FREW.—On *Chlorops taeniopus* Meig. (The Gout Fly of Barley). (pp. 175—219.)

EXPLANATION OF PLATES III AND IV

(For the photographs here reproduced I am indebted to Mr H. M. Morris.)

Plate III.

Fig. 1. A plant of winter barley showing the appearance of a shoot *A* attacked by gout fly compared with two normal shoots *B*.

Fig. 2. A shoot of couch grass attacked by the winter larva of *Chlorops taeniopus*.

Fig. 3. A normal shoot of couch grass during the winter.

Plate IV.

Fig. 1. The typical form of summer attack on barley.

Fig. 2. Ear and ear-bearing internode from a typical example of barley attacked by the summer generation of *Chlorops taeniopus*. *P* the puparium. Pupation has taken place in the food groove below the ear.

Fig. 3. Summer damage on barley resembling the winter form. *A* the attacked shoot; *B*, *C* and *D* unattacked shoots. *D* is a normal ear-bearing shoot which has been broken short.

Figs. 4 and 5. Non-typical forms of summer damage on barley.

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STATISTICAL STUDY ON THE EFFECT OF MANURING ON INFESTATION OF BARLEY BY GOUT FLY

By "MATHETES."

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The material supplied for this investigation consisted of the numbers of shoots injured in each of 10 samples of 100 taken from differently manured plots in various fields during 1922 as described in (4): the number of plots sampled is set out below, and the details are given in Appendix I.

Permanent Fields:	
Hoos Field	28
Woburn Stackyard Field	22
Malting Barley Experimental Fields:	
Long Hoos	7
Woburn	5
Dunmow (Mr Hasler's Farm)	5
Wellingore (Mr Nevil's Farm) ...	5

In order to determine whether there are significant differences between plots, corresponding to different manurial conditions, it was necessary first to ascertain whether the distribution of attack within the plots was random, in which case the mean infestation of any plot (see Appendix I) could be taken with confidence as a basis for comparison with other plots. Accordingly a preliminary survey was made along the lines developed successfully by Fisher (1) in determining the accuracy of the plating method of estimating the density of bacterial populations in the soil. If the numbers in the sub-samples be represented by

$$x_1, x_2, x_3, \ldots x_n,$$

so that $S(x) = n\bar{x}$, the index of dispersion

$$\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$$

can be calculated for each plot and if the samples can be represented as random samples from a Poisson series the distribution of the χ^2's should give a good fit when compared with the expected distribution taken from Elderton's tables entered with $n' = n$, the distribution of χ^2 being independent of $\bar{x}$.

In the present case we wish to test if the distribution agrees, not with the Poisson, but with the binomial distribution: this may be done with exactitude by modifying the formula for the index of dispersion. In such a case the formula for χ^2 becomes

$$\chi^2 = \frac{NS(\xi)^2}{(N-\bar{x})\bar{x}} = \frac{S(\xi)^2}{N\bar{p}\bar{q}},$$

where N is the number in the sub-sample (here = 100).

$$\xi = x - \bar{x}; \quad N\bar{p} = \bar{x};$$

$\bar{p}$ is the probability of attack and $\bar{q}$ of non-attack. The values of χ^2 were calculated from the above formula, and the correlation between mean infestation and χ^2 was found to be insignificant. The fit over the whole distribution was not very good, as the following table shows.

Table I.

The distribution of χ^2 for 70 plots compared with the expected distribution.

χ^2	Expected	Realised	Difference	d^2/E
< 3	2·52	1	−1·52	·9168
> 3 < 5	9·10	4	−5·10	2·8582
> 5 < 7	13·79	6	−7·79	4·4006
> 7 < 9	14·00	14	±0·00	—
> 9 <11	11·27	12	+ ·73	·0473
>11 <13	7·91	11	+3·09	1·2071
>13 <15	5·04	9	+3·96	3·1115
>15 <17	2·94	3	+ ·06	·0012
>17 <19	1·68	4	+2·32	3·2038
>19	1·75	6	+4·25	10·3217

$\chi^2 = 26{\cdot}0682$
$P = \cdot 002$

The ordinary test for goodness of fit shows the observed distribution to be more widely divergent from the expected than would be accounted for by the errors of random sampling. A more delicate test in such a case is to compare the total χ^2 with the calculated value, using the approximate formula $\sqrt{2n'} - 3$ to give P for values of n' outside the range of Elderton's tables. Here we find

$$S(\chi^2) = 790{\cdot}99$$
$$\sqrt{2\chi^2} = 39{\cdot}773$$
$$\sqrt{2n'} - 3 = 35{\cdot}548$$
$$\text{Difference} = 4{\cdot}225$$

This difference corresponds to the x/σ of the probability integral tables and being greater than 2 is definitely significant. The distribution

cannot, therefore, strictly speaking be regarded as consisting of random samples from a binomial series.

For individual fields we get the results shown in Table II.

Table II.

	Hoos		Woburn Stackyard		Long Hoos		Woburn		Dunmow		Wellingore	
χ^2	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised
< 3	1·008	—	·720	1	·252	—	·180	—	·180	—	·180	—
$> 3 < 5$	3·640	—	2·600	—	·910	2	·650	1	·650	—	·650	1
$> 5 < 7$	5·516	1	3·940	5	1·379	—	·985	—	·985	—	·985	—
$> 7 < 9$	5·600	5	4·000	3	1·400	1	1·000	2	1·000	1	1·000	2
$> 9 < 11$	4·508	5	3·220	4	1·127	—	·805	1	·805	2	·805	—
$> 11 < 13$	3·164	6	2·260	4	·791	1	·565	—	·565	—	·565	—
$> 13 < 15$	2·016	4	1·440	1	·504	1	·360	—	·360	1	·360	2
$> 15 < 17$	1·176	1	·840	—	·294	1	·210	1	·210	—	·210	—
$> 17 < 19$	·672	2	·480	1	·168	—	·120	—	·120	1	·120	—
> 19	·700	4	·500	1	·175	1	·125	—	·125	—	·125	—

which may be conveniently summarised thus:

Table III.

	Hoos		Woburn		Malting Barleys	
χ^2	Expected	Realised	Expected	Realised	Expected	Realised
< 3	10	1	7	6	8	4
$> 7 < 15$	15	20	11	12	12	14
> 15	3	7	2	2	2	4

In the case of Woburn Field and the Malting Barleys, the fit is sufficiently good to enable us to conclude that the sampling is random. In Hoos, however, the fit is anything but good, showing the intensity of attack to have varied within the plot more than is consistent with random sampling.

To test whether there were significant differences between plots, the distribution of χ^2 with $N = 1000$ and $\xi =$ mean of plot minus mean of field, compared with the expected value was examined. For instance, in the case of Woburn

$$\chi^2 = \frac{S(x - \bar{x})}{N\bar{p}\bar{q}} = 193{\cdot}9,$$

$n' = 20$, so that there were significant differences between plots. Such differences were found also in Hoos, Long Hoos and Wellingore, as the following table shows:

Table IV.

Expected and Realised Values of χ^2 over whole fields.

	χ^2	n'	Difference between plots
Hoos	548·043	28	Significant
Woburn	123·353	18 *	do.
	193·908	20 *	do.
Malting Barleys:			
Long Hoos	13·889	7	Significant
Woburn	5·359	5	Not significant
Dunmow	5·966	5	do.
Wellingore	30·093	5	Significant

* Two of the Woburn plots were very badly over-grown with weeds; the comparison is shown with and without these plots.

An interesting point in connection with Woburn Stackyard Field is that there was a marked indication that excessive soil acidity reduced infestation. On the very acid plots, however, the weeds were so numerous and the barley so scarce, that the infestation figures were rather suspect. It is a point which should be susceptible to experimental treatment, and for what they are worth, particulars are given of the very acid plots in Woburn, and their parallels where the acidity is reduced by the addition of lime.

Table V.

Plot No.	Soil *p*H value	Infestation	Treatment
2A	4·15	8·2	Sulphate of Ammonia (=25 lbs. NH_3)
2AA	5·01	16·3	do. +5 cwts. Lime 1905, 1909, 1910, 1912
5A	4·40	13·3	do. +Minerals
5AA	5·14	17·8	do. + do. +1 ton Lime 1905, 1916
8B	4·36	6·3	do. (alternate years) (=50 lbs. NH_3) +Minerals
8BB	6·61	16·9	do. +Minerals +2 tons Lime 1897, 1912

No great importance is attached to these figures; they are merely given as being suggestive and perhaps worthy of experimental investigation.

Having found that significant differences do exist between plots, the next step was to compare plots differing in one manure, so that if a significant difference is found, it can fairly be ascribed to the presence or absence of that manure. The method of comparison was in all cases the same, viz. if we have the following scheme:

	Number attacked	Number immune	
Superphosphate	a	b	$a+b$
No superphosphate	c	d	$c+d$
	$a+c$	$b+d$	$a+b+c+d$

Fisher has shown (2) that if

$$x = \frac{bc - ad}{(a+b)(c+d)}, \text{ then } \frac{x^2}{\sigma_x^2} = \frac{(bc-ad)^2(a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)} = \chi^2,$$

and that χ for $n' = 2$ is distributed over the positive half of a normal curve with unit standard deviation.

By determining $\chi = \sqrt{\frac{x^2}{\sigma_x^2}}$, we have at once a measure of significance: if $\chi > 2$, the difference is significant. As many comparisons as possible were made in this way, with the results set out below (*v.* Table VI). It should be noted here, that in the case of Hoos Field, since the mean χ^2 was about 25 per cent. higher than the expected value, the limit for significance in this case should be taken as 2·5 instead of 2·0.

The best set of comparisons is obtained from Hoos, but unfortunately very few comparisons are possible between the same pairs of manures in different fields, and even these do not to any great extent agree with the results for Hoos. This, of course, was to be expected, considering the previous history of the other fields compared with Hoos, where the plots have had the same manures for many years.

Considering the result for Hoos where any value of χ greater than 2·5 marks a significant effect of manure on infestation (+ indicates a beneficial effect), it will be seen that Superphosphate is almost invariably effective in reducing infestation, either alone or in conjunction with other manures. The only exceptions found were with Alkali Salts and Nitrate of Soda, and with the same combination + Silicate.

Farmyard Manure alone is very effective and so is Potash in presence of Superphosphate and Ammonium Salts.

Nitrate of Soda reduces infestation when acting alone, but not when used with Superphosphate, Alkali Salts or Minerals.

Ammonium Salts are only effective in conjunction with Minerals, or with Superphosphate and Potash.

Rape Cake alone has no effect, but reduces infestation when used with Superphosphate, Alkali Salts or Minerals.

Minerals either alone or with Ammonium Salts, Nitrate of Soda or Rape Cake, are beneficial; and Alkali Salts are only of service in presence of Nitrate of Soda or Rape Cake.

Hoos Field, on account of its previous history, is very valuable in any attempt to arrive at a quantitative estimate of the effects of different manures, and the remainder of the work, consisting of such an attempt, dealt entirely with Hoos. The idea was to determine for each manure

Table VI. *Values of χ for fourfold manurial comparisons.*

	Permanent Barley		Long Hoos	Wellingore	Long Hoos Woburn Wellingore Dunmow
	Hoos	Woburn			
Superphosphate *v.* unmanured	+3·522				
,, with Rape Cake	+5·859				
,, ,, Ammonium Salts (+Potash)	+3·577	—	+1·022	−5·621	−1·076
,, ,, Sodium Nitrate	+2·688	−2·364			
,, ,, Alkali Salts	+3·303				
,, ,, ,, +Ammonium Salts	+8·787				
,, ,, ,, +$NaNO_3$ ...	+1·699				
,, ,, $NaNO_3$ +Silicate ...	+3·914				
,, ,, ,, + ,, +Alkali Salts	+2·084				
,, ,, Rape Cake +Alkali Salts ...	+3·782				
Farmyard Manure *v.* unmanured	+7·434	+4·560			
Potash with $NaNO_3$	—	+1·317			
,, ,, Superphosphate	+3·400				
,, ,, ,, +Ammonium Salts	+6·625	—	+2·704	−2·001	+ ·802
Sodium Nitrate *v.* unmanured	+4·139	25 lbs. NH_3 +4·491 50 lbs. NH_3 +3·465			
,, ,, with Superphosphate	+1·783				
,, ,, ,, Alkali Salts	+2·259				
,, ,, ,, Minerals†	+1·354	− ·060			
Ammonium Salts *v.* unmanured	− ·463	+8·909			
,, ,, with Superphosphate ...	+ ·066				
,, ,, ,, Alkali Salts	−1·490				
,, ,, ,, Minerals	+4·262	+5·317*			
,, ,, ,, Superphosphate +Potash	+3·476	—	+ ·834	−2·266	− ·992
Rape Cake *v.* unmanured	− ·332	+2·994			
,, ,, with Superphosphate	+2·514				
,, ,, ,, Alkali Salts	+4·178				
,, ,, ,, Minerals	+4·642				
Minerals *v.* unmanured	+5·507	+2·994			
,, with Ammonium Salts	+9·311	−1·425*			
,, ,, $NaNO_3$	+4·013				
,, ,, Rape Cake	+3·019				
Alkali Salts *v.* unmanured	+1·900				
,, ,, with $NaNO_3$	+2·671				
,, ,, ,, Ammonium Salts	+ ·574				
,, ,, ,, Rape Cake	+6·077				
Sodium Silicate with $NaNO_3$	−1·905				
,, ,, ,, ,, +Alkali Salts ...	+ ·767				
,, ,, ,, ,, +Superphosphate ...	+ ·145				
,, ,, ,, ,, +Minerals ...	+1·226				
Lime with $NaNO_3$	—	25 lbs. NH_3 + ·517 50 lbs. NH_3 − ·725			
,, ,, Minerals	—	−1·177			
,, ,, Ammonium Sulphate	—	−5·524			

* Include plots overgrown with weeds.

† Minerals in the case of Hoos means Potash, Superphosphate, Sodium and Magnesium Sulphates. The same was true of Woburn until 1905, since when it has meant Superphosphate and Potash only.

a figure showing its efficiency in reducing infestation, and thus form an equation to predict the infestation on any plot solely from a consideration of the manurial treatment. The mathematical theory of the method employed has been developed by Fisher (3), and was applied in the following way. From the percentages immune, and the number of observations on 26 of the Hoos plots (Farmyard Manure and ashes plots omitted), weights were obtained by the method of (3), and from the 26 observational equations, seven normal equations derived, the solution of which gave the values of the seven unknowns:

q_O representing the % immunity conferred by no manures.
q_R representing the additional % immunity conferred by Rape Cake
q_A " " " " " Ammonium Sulphate
q_N " " " " " Nitrate of Soda
q_S " " " " " Sodium and Magnesium Sulphate
q_K " " " " " Potash
q_P " " " " " Superphosphate

The following table shows the arrangement of the work:

Table VII.

Plot No.	Manurial treatment	% immune	No. immune (x)	No. of observations (n)	n^2/y	n^3/xy	Suffixes involved
7.1	Unmanured after Dung	81·8	818	1000	5494·5	6717·03	O
6.1	Unmanured	80·9	809	"	5235·6	6471·73	O
1.0	Unmanured	82·1	821	"	5586·6	6804·48	O
2.0	Superphosphate	86·6	866	"	7462·7	8617·18	O P
3.0	Alkali Salts	84·3	843	"	6369·4	7555·38	O P S
4.0	Complete Minerals	89·3	893	"	9345·8	10465·43	O P K S
5.0	Superphosphate + Potash	91·3	913	"	11494·0	12589·38	O P K
1A	Ammonium Salts	80·8	808	"	5208·3	6445·79	O A
2A	do. + Superphosphate	86·7	867	"	7518·8	8672·18	O A P
3A	do. + Alkali Salts	81·8	818	"	5494·5	6717·03	O A S K
4A	do. + Complete Minerals ...	94·5	945	"	18182·0	19240·19	O A K P S
5A	do. + Potash + Superphosphate ...	95·2	952	"	20833·0	21882·98	O A K P
1AA	Sodium Nitrate	80·5	805	"	5128·2	6370·25	O N
2AA	do. + Superphosphate ...	89·2	892	"	9259·3	10380·60	O N P
3AA	do. + Alkali Salts	87·8	878	"	8196·7	9336·04	O N K S
4AA	do. + Complete Minerals ...	91·1	911	"	11236·0	12333·76	O N K P S
1AAS	do. + Sodium Silicate	83·4	834	"	6024·1	7222·90	O N
2AAS	do. + do. + Superphosphate ...	89·4	894	"	9434·0	10552·87	O N P
3AAS	do. + do. + Alkali Salts ...	88·9	889	"	9090·9	10226·35	O N K S
4AAS	do. + do. + Complete Minerals	92·6	926	"	13514·0	14593·77	O N K P S
1C	Rape Cake	81·0	810	"	5263·2	6497·95	O R
2C	do. + Superphosphate	90·2	902	"	10204·0	11312·15	O R P
3C	do. + Alkali Salts	90·5	905	"	10526·0	11631·23	O R K S
4C	do. + Complete Minerals ...	94·9	949	"	19608·0	20660·95	O R K P S
1N	Sodium Nitrate	87·0	870	"	7692·3	8841·53	O N
2N	Sodium Nitrate	90·1	901	"	10101·0	11211·10	O N

From this we derive the following Normal Equations:

$$273350{\cdot}23\,q_O + 168856{\cdot}82\,q_P + 149677{\cdot}11\,q_K + 122760{\cdot}13\,q_S + 101069{\cdot}17\,q_N + 62958{\cdot}17\,q_A + 50102{\cdot}28\,q_R = 243502{\cdot}9$$
$$168856{\cdot}82\,q_O + 168856{\cdot}82\,q_P + 111766{\cdot}46\,q_K + 84849{\cdot}48\,q_S + 47861{\cdot}00\,q_N + 49795{\cdot}35\,q_A + 31973{\cdot}10\,q_R = 154461{\cdot}0$$
$$149677{\cdot}11\,q_O + 111766{\cdot}46\,q_P + 149677{\cdot}11\,q_K + 115204{\cdot}75\,q_S + 46489{\cdot}92\,q_N + 47840{\cdot}20\,q_A + 32292{\cdot}18\,q_R = 137520{\cdot}9$$
$$122760{\cdot}13\,q_O + 84849{\cdot}48\,q_P + 115204{\cdot}75\,q_K + 122760{\cdot}13\,q_S + 46489{\cdot}92\,q_N + 25957{\cdot}22\,q_A + 32292{\cdot}18\,q_R = 111563{\cdot}3$$
$$101069{\cdot}17\,q_O + 47861{\cdot}00\,q_P + 46489{\cdot}92\,q_K + 46489{\cdot}92\,q_S + 101069{\cdot}17\,q_N + \text{—} + \text{—} = 89676{\cdot}5$$
$$62958{\cdot}17\,q_O + 49795{\cdot}35\,q_P + 47840{\cdot}20\,q_K + 25957{\cdot}22\,q_S + \text{—} + 62958{\cdot}17\,q_A + \text{—} = 57236{\cdot}6$$
$$50102{\cdot}28\,q_O + 31973{\cdot}10\,q_P + 32292{\cdot}18\,q_K + 32292{\cdot}18\,q_S + \text{—} + \text{—} + 50102{\cdot}28\,q_R = 45601{\cdot}2$$

The solution of the normal equations yielded the values

$q_O = \cdot 811000$	$q_S = -\cdot 023727$
$q_R = \cdot 042208$	$q_K = \cdot 061439$
$q_A = \cdot 020737$	$q_P = \cdot 051159$
$q_N = \cdot 034363$	

whence we can place the manures in the order of their efficiency in decreasing infestation as follows:

Complete Minerals	8·9 %
Potash	6·1
Superphosphate	5·1
Rape Cake	4·2
Alkali Salts (Potash + Sodium and Magnesium Sulphates)	3·8
Nitrate of Soda	3·4
Ammonium Salts	2·1
Sodium and Magnesium Sulphates	−2·3

Still following the method of (3), the percentage immunity was calculated for each plot from these values, and the differences between the actual and calculated values compared with the standard error, with the following results:

Table VIII.

Actual	Calculated	Difference (d)	Standard error (σ)	d^2/σ^2
81·8	81·1	+ ·7	1·2	·34
80·9	81·1	−1·1	1·2	·84
82·1	81·1	+1·0	1·2	·69
86·6	86·2	+ ·4	1·1	·13
84·3	84·9	− ·6	1·1	·30
89·3	90·0	− ·7	·9	·60
91·3	92·3	−1·0	·8	1·56
80·8	83·2	−2·4	1·2	4·00
86·7	88·3	−1·6	1·0	2·56
81·8	87·0	−5·2	1·1	22·30
94·5	92·1	+2·4	·9	7·12
95·2	94·4	+ ·8	·7	1·31
80·5	84·5	−4·0	1·1	13·24
89·2	89·6	− ·4	1·0	·16
87·8	88·3	− ·5	1·0	·25
91·1	90·0	+1·1	·9	1·49
83·4	84·5	−1·1	1·1	1·00
89·4	89·6	− ·2	1·0	·04
88·9	88·3	+ ·6	1·0	·36
92·6	93·4	− ·8	·8	1·00
81·0	85·3	−4·3	1·1	15·28
90·2	90·4	− ·2	·9	·05
90·5	89·1	+1·4	1·0	1·96
94·9	94·2	+ ·7	·7	1·00
87·0	84·5	+2·5	1·1	5·17
90·1	84·5	+5·6	1·1	25·09

$\chi^2 = 107 \cdot 84.$

It will be seen that the majority of the results show good agreement; in only six plots was there a very wide divergence of observed and calculated, three of which were Nitrate plots alone, and one Rape Cake alone. It may be noted in passing that the Nitrate plots were so discordant that no theory could cover them. The value of χ^2 from d^2/σ^2 was 107·84 (mostly accounted for by the six bad plots) and comparing this with the value of χ^2 for the whole field, having regard to the number of degrees of freedom used, we have

$$\frac{107{\cdot}84}{19} : \frac{548{\cdot}04}{27} = 5{\cdot}66 : 20{\cdot}30,$$

and allowing $\chi^2 = 1$ for each, this reduces to 4·66 : 19·3. That is to say, we have accounted for 76 per cent. of the variance on the basis of a linear arrangement of the manures as regards their efficiency in reducing infestation; of the remaining 24 per cent., $6\frac{1}{2}$ per cent. is ascribable to sampling; and considering that the 107·84 was mainly due to six exceptional plots, this result may be regarded as satisfactory, and furnishing fairly conclusive evidence that manures have a differential and quantitatively measurable effect in reducing gout fly infestation of barley.

As to whether we have been measuring the effect of the manures in conferring immunity on the plant by

(1) transference of some of its constituents into the plant, and so

(*a*) causing a hardening of the plant tissues so as to render the passage of the larvae more difficult, or

(*b*) rendering the plant tissues unpalatable, or

(2) by promoting early growth, it was impossible to say without further investigation, and this has now been carried out by Mr Frew who has discussed the whole question in (4).

For the purposes of this paper, it is only necessary to discuss confirmatory work carried out during 1923.

INFESTATION OF BARLEY IN HOOS FIELD, 1923.

A series of counts similar to that of 1922 was carried out in 1923, but with a few additional particulars, such as estimates of shoot density, etc. (*v.* Appendix II) and as it is of some interest to know how far the later results confirm the earlier, a brief survey is appended.

The observed and expected distribution of χ^2 is set out in Table IX.

Table IX. *Distribution of χ^2 for Hoos Field* 1923.

χ^2	Expected	Observed
< 3	1·008	1
$> 3 < 5$	3·640	4
$> 5 < 7$	5·516	5
$> 7 < 9$	5·600	3
$> 9 < 11$	4·508	6
$> 11 < 13$	3·164	4
$> 13 < 15$	2·016	2
$> 15 < 17$	1·176	—
$> 17 < 19$	·672	—
$> 19 < 21$	·336	1
> 21	·362	2
	28·000	

Testing the goodness of fit we find

$$\sqrt{2\chi^2} = 23{\cdot}907$$
$$\sqrt{2n' - 3} = 22{\cdot}428$$
$$\text{Difference} = 1{\cdot}479$$

showing the fit to be good, and since the correlation between mean infestation and χ^2 is insignificant, we are justified in regarding our samples as random samples from a binomial distribution.

For the whole field $\chi^2 = 561{\cdot}84$ and as $n' = 28$, it is obvious that there are significant differences between plots.

Correlating the infestation means of the plots in 1922 with those in 1923, we find $r = + {\cdot}635$, showing about 64 per cent. of the causes of variation in degree of infestation to be common to both years. All things considered, this is probably as good agreement as one could reasonably expect in experiments of this nature, and may fairly be held as confirming our conclusion as to the differential effect of manures on infestation.

Among the additional counts made in 1923 were those of shoot density, or number of shoots per given area, and number of infested shoots for the same area, in order to test the hypothesis that if the attack is absolutely at random over the whole field, those plots with the greatest shoot density would tend to have a diminished infestation.

The coefficient of correlation showing the relationship between shoot density and number of infested shoots per square yard is $r = - {\cdot}39$. Having regard to the small number of cases (28) this is barely significant; it indicates that increased shoot density does in fact tend to be associated with reduced infestation, but not sufficiently so to make it an important cause of variation in the latter.

The results of the fourfold manurial comparisons are set out in Table X, for comparison with Table VI.

Table X.

Values of χ for fourfold Manurial Comparisons. Hoos Field, 1923.

Superphosphate *v.* unmanured	+6·371
„ with Rape Cake	+ ·934
„ „ Ammonium Salts (+Potash) ...	+5·000
„ „ Sodium Nitrate	+8·330
„ „ Alkali Salts	+1·670
„ „ „ „ +Ammonium Salts ...	+7·272
„ „ „ „ +Sodium Nitrate ...	+7·800
„ „ Sodium Nitrate+Silicate	+6·740
„ „ „ „ + „ +Alkali Salts	+7·070
„ „ Rape Cake+Alkali Salts	+1·089
Farmyard Manure *v.* unmanured	+7·327
Potash with Superphosphate	−3·809
„ „ „ „ +Ammonium Salts	−1·212
Sodium Nitrate *v.* unmanured	−2·125
„ „ with Superphosphate	+ ·407
„ „ „ Alkali Salts	−3·478
„ „ „ Minerals	+2·908
Ammonium Salts *v.* unmanured	−1·066
„ „ with Superphosphate	−2·094
„ „ „ Alkali Salts	−2·911
„ „ „ Minerals	+2·908
„ „ „ Superphosphate+Potash	+ ·535
Rape Cake *v.* unmanured	+5·172
„ „ with Superphosphate	− ·296
„ „ „ Alkali Salts	+2·096
„ „ „ Minerals	+1·515
Minerals *v.* unmanured	+4·280
„ with Ammonium Salts	+7·450
„ „ Sodium Nitrate	+8·692
„ „ Rape Cake	+ ·647
Alkali Salts *v.* unmanured	+3·513
„ „ with Sodium Nitrate	+ ·178
„ „ „ Ammonium Salts	+ ·193
„ „ „ Rape Cake	− ·443
Sodium Silicate with Sodium Nitrate	+ ·644
„ „ „ „ „ +Alkali Salts ...	+ ·190
„ „ „ „ „ +Superphosphate ...	+ ·306
„ „ „ „ „ +Minerals	− ·628

It will be seen that on the whole there is fairly good agreement between the two years' results, the most striking change being that Potash does not appear to have exercised any beneficial effect in 1923. Minerals, Superphosphate, and Farmyard Manure are again markedly beneficial, and it seems highly probable that a more detailed examination of the 1923 results, which lack of time forbids my carrying out, would yield efficiency figures for the various manures (with the exception of Potash) not widely different from those found in 1922. At any rate

there is no doubt about the main result, that in any attempt to reduce gout fly infestation on such land as at Rothamsted by means of the application of manures, the best results will be obtained by Farmyard Manure, complete Minerals or Superphosphate. For a full discussion as to the way in which these manures act in bringing about a reduction in infestation, the reader is referred to Mr Frew's paper (4).

In conclusion, I have to acknowledge my indebtedness to Mr R. A. Fisher, in whose laboratory this work was done, and who contributed invaluable help and advice throughout.

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Appendix I.

Table showing manurial treatment, number of shoots attacked per hundred examined, mean percentage infestation, and χ^2.

Data for Hoos Field. Barley, 1922.

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
7·1	Unmanured after Dung 1852–1871 ...	... 18, 26, 18, 20, 16, 14, 17, 27, 13, 13	18·2	14·76
7.2	Farmyard Manure	... 8, 3, 4, 14, 11, 7, 16, 7, 5, 7	8·2	21·47
6.1	Unmanured	... 24, 22, 23, 27, 20, 13, 14, 16, 17, 15	19·1	13·25
6.2	20 bushels Ashes	... 20, 19, 17, 20, 13, 31, 13, 11, 23, 17	18·4	20·13
1.0	No Minerals and no N (*i.e.* unmanured)	... 13, 25, 17, 17, 18, 21, 21, 21, 9, 17	17·9	12·58
2.0	Superphosphate only. No. N ...	... 15, 11, 12, 16, 15, 12, 17, 7, 16, 13	13·4	7·10
3.0	Alkali Salts only. No. N	... 19, 16, 11, 11, 20, 20, 15, 20, 16, 9	15·7	11·79
4.0	Complete Minerals. No. N ...	... 15, 9, 6, 12, 11, 19, 12, 9, 6, 8	10·7	15·50
5.0	Superphosphate + Potassium Sulphate	... 10, 14, 8, 6, 5, 12, 7, 9, 6, 10	8·7	9·33
1A	Ammonium Salts alone	... 20, 16, 24, 19, 20, 19, 20, 28, 11, 15	19·2	12·34
2A	Superphosphate + Ammonium Salts	... 13, 9, 12, 15, 18, 12, 19, 11, 10, 14	13·3	8·34
3A	Alkali Salts + Ammonium Salts ...	... 16, 14, 20, 20, 19, 22, 23, 14, 11, 23	18·2	10·72
4A	Complete Minerals + Ammonium Salts	... 4, 4, 6, 12, 2, 10, 5, 5, 7, 0	5·5	21·64
5A	Potassium Sulphate + Superphosphate + Ammonium Salts	... 4, 6, 2, 7, 8, 3, 4, 7, 4, 3	4·8	8·22
1AA	Sodium Nitrate alone	... 21, 19, 15, 13, 31, 22, 19, 18, 22, 15	19·5	14·81
2AA	Superphosphate + Sodium Nitrate ...	... 16, 7, 4, 13, 7, 13, 12, 14, 11, 11	10·8	12·83
3AA	Alkali Salts + Sodium Nitrate ...	... 12, 11, 14, 8, 19, 15, 14, 11, 9, 9	12·2	9·49
4AA	Complete Minerals + Sodium Nitrate	... 8, 10, 11, 12, 14, 6, 11, 7, 7, 3	8·9	11·95
1AAS	As 1AA + Sodium Silicate	... 19, 14, 21, 15, 14, 23, 13, 15, 16, 16	16·6	7·34
2AAS	As 2AA + Sodium Silicate	... 13, 8, 10, 11, 11, 7, 7, 10, 15, 14	10·6	7·43
3AAS	As 3AA + Sodium Silicate	... 8, 9, 18, 9, 14, 11, 9, 16, 9, 8	11·1	11·84
4AAS	As 4AA + Sodium Silicate	... 12, 10, 8, 1, 4, 5, 4, 7, 10, 13	7·4	19·90
1C	Rape Cake alone	... 20, 22, 15, 25, 10, 25, 21, 15, 21, 16	19·0	13·78
2C	Superphosphate + Rape Cake ...	... 10, 11, 10, 9, 8, 7, 10, 16, 6, 9	9·8	6·74
3C	Alkali Salts + Rape Cake	... 10, 9, 6, 12, 13, 13, 11, 8, 10, 3	9·5	10·53
4C	Complete Minerals + Rape Cake ...	... 8, 3, 2, 3, 4, 4, 6, 8, 11, 2	5·1	17·13
1N	Sodium Nitrate alone	... 6, 9, 10, 13, 10, 12, 14, 23, 16, 17	13·0	18·56
2N	Sodium Nitrate alone	... 4, 9, 10, 9, 9, 13, 15, 13, 8, 9	9·9	9·74

2N had double the quantity of Sodium Nitrate up to 1857; since then the 1N and 2N plots have had equal quantities.

Data for Woburn. Barley (Stackyard Field).

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
10A	Superphosphate 3 cwts. + Sodium Nitrate (=25 lbs. Ammonia)	21, 26, 19, 18, 10, 14, 22, 20, 15, 17	18·2	12·33
10B	Rape Cake (=25 lbs. Ammonia)	15, 22, 20, 17, 14, 12, 13, 15, 17, 20	16·5	7·15
11A	Sulphate of Potash 1 cwt. + Sodium Nitrate (=25 lbs. Ammonia)	9, 11, 20, 18, 8, 15, 17, 9, 11, 5	12·3	20·22
11B	Farmyard Manure (=100 lbs. Ammonia)	16, 11, 11, 12, 13, 19, 14, 14, 14, 18	14·2	5·55
9B	Mineral Manures and in alternate years Sodium Nitrate (=50 lbs. Ammonia) (applied 1922)	11, 14, 14, 14, 12, 13, 15, 13, 18, 21	14·5	6·33
6	Mineral Manures and Sodium Nitrate (=25 lbs. Ammonia)	19, 14, 18, 17, 17, 16, 10, 13, 21, 19	16·4	7·03
3B	(End limed in 1921) Sodium Nitrate (=25 lbs. Ammonia)	19, 16, 13, 14, 8, 10, 11, 15, 14, 15	13·5	7·75
,,	(End not limed in 1921) ,, ,, (,, ,,)	15, 11, 14, 16, 20, 14, 16, 12, 10, 15	14·3	6·04
3A	(End limed in 1921) ,, ,, (=50 lbs. Ammonia)	16, 18, 11, 18, 21, 10, 20, 18, 17, 21	17·0	9·22
,,	(End not limed in 1921) ,, ,, (,, ,,)	12, 21, 13, 14, 14, 17, 22, 16, 18, 11	15·8	9·29
5A	Mineral Manures + Sulphate of Ammonia (=25 lbs. Ammonia)	17, 17, 13, 12, 16, 7, 7, 14, 18, 12	13·3	12·15
5AA	As 5A but with 1 ton of Lime March 1905; repeated April 1916	14, 17, 28, 17, 17, 16, 12, 24, 15, 18	17·8	13·92
8B	Mineral Manures and in alternate years Sulphate of Ammonia (=50 lbs. Ammonia) (applied 1922)	5, 6, 7, 5, 8, 13, 3, 5, 6, 5	6·3	11·20
8BB	As 8B but with 2 tons of Lime December 1897; repeated 1912	18, 18, 17, 17, 18, 16, 17, 18, 15, 15	16·9	·91
2A	Sulphate of Ammonia (=25 lbs. Ammonia)	9, 4, 12, 13, 8, 11, 3, 7, 7, 8	8·2	12·43
2AA	As 2A but with 5 cwts. Lime March 1905; repeated 1909, 1910 and 1912 ...	9, 20, 15, 14, 19, 17, 18, 22, 18, 11	16·3	10·86
4A	Mineral Manures alone	12, 20, 14, 16, 17, 19, 20, 17, 19, 11	16·5	6·86
4B	As 4A but with 1 ton Lime March 1915	25, 21, 23, 11, 12, 15, 19, 22, 25, 12	18·5	18·34
7	No Manure	24, 23, 23, 18, 21, 15, 24, 30, 24, 17	21·9	9·87
1	No Manure	17, 22, 20, 27, 19, 22, 20, 23, 16, 17	20·3	6·19

Malting Barley Experimental Plots.

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
1. *Long Hoos Field, Rothamsted.*				
1	3 cwt. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	14, 18, 14, 9, 13, 12, 10, 11, 15, 19	13·5	8·09
2	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	17, 16, 16, 10, 16, 14, 19, 16, 12, 15	15·1	4·51
3	3 cwts. Superphosphate + 1 cwt. Sulphate of Ammonia	23, 26, 15, 15, 23, 12, 13, 14, 15, 23	17·9	16·53
4	„ „ + 1½ cwts. Sulphate of Potash	12, 13, 15, 18, 13, 13, 18, 14, 23, 9	14·8	11·07
5	No Manure	11, 16, 13, 12, 8, 15, 13, 13, 14, 14	12·9	4·19
6	3 cwts. Superphosphate + 1½ cwts. Muriate of Potash + 1 cwt. Sulphate of Ammonia	12, 21, 6, 18, 17, 19, 12, 10, 9, 5	13·2	24·50
7	„ „ + 1½ cwts. Sulphate of Potash + 93 lbs. Muriate of Ammonia	16, 15, 9, 12, 16, 12, 19, 23, 12, 10	14·4	13·50
2. *Woburn Malting Barley.*				
1	No Manure	10, 10, 13, 7, 7, 4, 9, 8, 11, 7	8·6	7·43
2	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	10, 15, 8, 6, 8, 14, 13, 10, 10, 9	10·3	8·02
3	„ „ + 1½ cwts. Sulphate of Potash	10, 13, 9, 4, 2, 15, 9, 11, 8, 9	9·0	16·12
4	„ „ + 1 cwt. Sulphate of Ammonia	13, 8, 13, 15, 11, 9, 12, 13, 10, 9	11·3	4·60
5	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	13, 15, 12, 10, 11, 7, 10, 11, 4, 10	10·3	9·11
3. *Dunmow Malting Barley (Mr Hasler's Farm).*				
1	No Manure	4, 7, 3, 7, 7, 2, 6, 2, 4, 7	4·9	8·78
2	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	7, 2, 5, 4, 6, 5, 1, 2, 7, 7	4·6	10·58
3	„ „ + 1½ cwts. Sulphate of Potash	3, 5, 4, 2, 5, 7, 2, 1, 10, 2	4·1	17·52
4	„ „ + 1 cwt. Sulphate of Ammonia	2, 4, 3, 1, 4, 0, 4, 2, 6, 3	2·9	9·56
5	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	1, 1, 3, 5, 7, 8, 3, 8, 3, 5	4·4	14·94
4. *Wellingore Malting Barley (Mr Nevil's Farm).*				
A	1 cwt. Sulphate of Ammonia + 1½ cwts. Sulphate of Potash	1, 0, 1, 1, 1, 0, 1, 0, 0, 1	·6	4·02
B	No Manure	1, 3, 2, 5, 3, 3, 0, 4, 2, 3	2·6	7·27
C	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	4, 7, 4, 7, 6, 6, 6, 2, 2, 2	4·6	8·75
D	„ „ + 1½ cwts. Sulphate of Potash	0, 3, 2, 0, 2, 1, 4, 4, 6, 5	2·7	14·50
E	„ „ + 1 cwt. Sulphate of Ammonia	3, 3, 4, 3, 0, 3, 2, 1, 2, 8	2·9	14·52

APPENDIX II.

Data for Hoos Field. Barley, 1923.

(Manurial treatment as in Appendix I.)

Plot No.	Shoots attacked per 100 examined	Mean infestation %	χ^2	Infested shoots per sq. yd.	No. of shoots per 2 sq. yds. at time of infestation counts
7.1	14, 24, 21, 11, 11, 17, 23, 13, 16, 18	16·8	14·28	47·1	561
7.2	1, 4, 3, 5, 9, 4, 4, 3, 5, 5	4·3	9·26	16·4	763
6.1	11, 19, 17, 12, 14, 12, 10, 14, 15, 17	14·1	6·35	33·7	478
6.2	14, 11, 14, 12, 15, 20, 10, 14, 14, 16	14·0	5·81	37·9	541
1.0	11, 11, 15, 5, 12, 11, 12, 10, 15, 13	11·5	7·12	28·0	487
2.0	7, 5, 4, 6, 3, 6, 4, 6, 6, 6	5·3	2·81	15·7	594
3.0	8, 10, 6, 8, 12, 8, 12, 7, 9, 17	9·7	10·74	24·6	508
4.0	6, 5, 8, 5, 6, 10, 5, 10, 7, 14	7·6	11·16	19·6	516
5.0	8, 18, 10, 9, 6, 10, 12, 8, 10, 7	9·8	11·49	24·1	493
1A	16, 20, 14, 10, 16, 17, 14, 13, 11, 11	14·2	7·19	36·6	516
2A	5, 8, 4, 4, 9, 14, 6, 8, 7, 11	7·6	12·87	24·6	659
3A	7, 18, 16, 21, 13, 15, 13, 13, 12, 11	13·9	11·27	41·7	600
4A	5, 6, 11, 3, 4, 4, 2, 2, 5, 3	4·5	14·53	14·1	628
5A	8, 3, 10, 16, 13, 5, 14, 6, 6, 10	9·1	19·70	22·1	486
1AA	11, 14, 15, 22, 18, 12, 18, 16, 16, 23	16·5	9·91	49·3	597
2AA	4, 3, 8, 4, 5, 7, 6, 4, 2, 6	4·9	6·63	17·8	727
3AA	11, 13, 10, 11, 18, 21, 15, 15, 18, 16	14·8	9·17	44·7	604
4AA	5, 4, 2, 2, 13, 3, 2, 6, 1, 7	4·5	26·63	13·3	589
1AAS	12, 12, 19, 17, 12, 13, 9, 12, 16, 19	14·1	8·66	42·2	598
2AAS	6, 2, 9, 5, 6, 6, 6, 4, 5, 3	5·2	6·82	17·5	673
3AAS	14, 15, 16, 16, 17, 6, 11, 14, 19, 17	14·5	9·88	36·6	574
4AAS	11, 1, 7, 6, 7, 2, 3, 2, 12, 0	5·1	32·42	18·8	736
1C	7, 8, 6, 4, 7, 6, 4, 8, 8, 8	6·6	3·64	20·1	609
2C	5, 3, 7, 7, 6, 4, 7, 6, 7, 4	5·6	3·86	18·2	652
3C	5, 3, 7, 9, 7, 8, 10, 7, 8, 7	7·1	5·29	21·7	611
4C	3, 4, 9, 6, 2, 8, 8, 7, 4, 8	5·9	9·89	18·6	629
1N	14, 14, 16, 14, 11, 10, 15, 10, 12, 13	12·9	3·46	37·8	586
2N	13, 14, 12, 16, 20, 14, 19, 16, 17, 13	15·4	4·94	40·8	530

THE ACTION OF SOME ORGANIC COMPOUNDS UPON THE CUCUMBER-HOUSE WOODLOUSE

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(With 1 Text-figure.)

In the search for a good means of controlling the Woodlouse (*Armadillidium speyeri* Jackson) which infests cucumber houses, and which attacks the cucumber plant to a serious extent, the action of a number of aromatic compounds has been tested, with a view to obtaining some substance which would kill these Crustacea in the soil during the winter months.

It is a habit of the woodlice to bury themselves in heaps of soil near glasshouses in the winter, and they are thus brought into the houses in great numbers, during the growing season, in soil used for propagating seed and for making up borders.

To ensure a true standard of comparison, solid substances were chosen throughout. In the experiments an attempt was made to distinguish between contact and vapour action. Owing to the possibility of a vapour condensing on the surface of the animal, these two effects could in no case be sharply differentiated. It was clear that death of the woodlice was not produced in the same way by chemically allied compounds, so that no relation between chemical constitution and action on the animal is to be expected. The results of the experiments, indeed, give us reason to believe that no such correlation exists.

Three sets of conditions were employed in testing for the action of each compound.

In the first, the woodlice came into direct contact with the pure substance; in the second, direct contact was avoided so that the woodlice were exposed to the vapour. In the third, the woodlice were kept in soil with which each substance had been intimately mixed.

A list of the compounds used will be found in the experimental portion of this paper. The most interesting phenomena shown by the experiments were:

(1) The extreme rapidity of death by contact and vapour when

Phenol and *o*-Cresol were used, and the great length of time over which both were operative when mixed with soil, especially the former.

(2) The slow action of the para- as compared with corresponding ortho-compounds used.

(3) The effects of different compounds on different parts of the animal tissues, some affecting the nervous system, others the respiratory system (*e.g.* Hydroquinone), another (Camphene) causing rapid death only by contact.

(4) The greater resistance of some individuals, as compared with others, to the action of any one substance, especially in the case of slow-acting compounds such as Hydroquinone, *p*-Nitrophenol, or Camphor.

(5) The greatest activity of several compounds, *e.g.* Hydroquinone and Camphor, being shown shortly before their disappearance from the soil with which they have been mixed.

(6) The rapid disappearance of Naphthalene when mixed with soil, and the prolonged action of *α*-Naphthylamine under similar conditions.

When subjected to the action of a substance which has a deleterious effect upon them, the woodlice pass through a more or less prolonged period of paralysis, losing control of the flexor and extensor muscles of the body. When paralysis is complete in the latter, the legs and antennae continue to twitch for a considerable time: if removed to normal conditions in this state revival may sometimes take place, but no case has been observed in which a woodlouse recovered after all movement had ceased.

In the case of *α*-Naphthylamine the period of paralysis before death was reduced to a minmium, being often practically unrecognisable. It is not always possible to distinguish between a respiratory and a nervous action, but where death was preceded by a phase in which the gill-plates became brown or blackish in colour, where the abdominal segments were much extended, and the outer division of the tail-appendages were turned upwards, it was concluded that death finally occurred through derangement of the respiratory system. Where the quicker acting substances were used, it appeared that the nervous system became disordered through the nerve-endings in the carapace, and upon the surface of the animal in general. With regard to the slow action of many compounds when mixed with soil, it is evident that a considerable time elapses before the soil-particles become generally invested with the compound in question, and in some cases this might closely precede the time at which the substance begins to disappear.

Further, it is at this period of saturation when the woodlice would be likely to come most often into contact with minute particles of the

compound, resulting in their more rapid death. That such contact does occur is clearly evident by the action of both ortho- and para-nitrophenol, where the thoracic legs and gill-plates are stained yellow upon the surface which actually comes into contact with the soil most frequently.

The length of time for which any substance remains operative in affecting woodlice in the soil has been tested by introducing the animals into such soil at definite times after mixing. The results have been standardised from the number of deaths amongst a fixed number of woodlice during a period of 24 hours. The comparative persistency of the most active substances mixed in equivalent proportions in the soil will readily be seen from the curves in the figure.

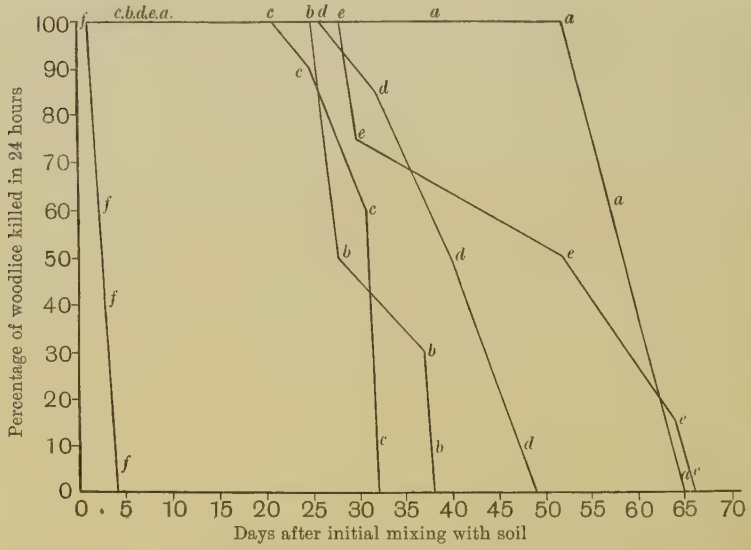

Fig. 1. Curves showing the duration of action on woodlice of substances when mixed with soil: (*a*) phenol, (*b*) *o*-cresol, (*c*) *p*-cresol, (*d*) *o*-nitrophenol, (*e*) α-naphthylamine, (*f*) naphthalene.

Each of the compounds illustrated killed, in the experiments, 100 per cent. of the woodlice introduced in a 24 hours' period of time:

Phenol	for 52 days	*o*-Nitrophenol	for 26 days
α-Naphthylamine	„ 28 „	*o*-Cresol	„ 25 „
Naphthalene	„ 3 „	*p*-Cresol	„ 20 „

All the compounds are injurious to plant growth, and must therefore be allowed to disappear from the soil before sowing or planting. In the case of Phenol, it has been found that tomato plants will germinate

practically normally if the seed is sown 20 days after mixing at the rate of 1 part Phenol in 750 parts of soil. This amount is sufficient to kill 100 per cent. woodlice for at least six days after mixing.

EXPERIMENTAL.

The compounds were tested in three different ways upon the woodlice, the following actions being observed. (All compounds tested were solid.)

(*a*) Action by direct contact.

(*b*) Action by vapour.

(*c*) Action of the substance when intimately mixed with the soil.

In testing (*a*), a small amount of each substance was placed at the bottom of a wide glass tube, and five woodlice dropped in, the tube being left open at the mouth. The experiments were made at room temperature.

In (*b*), similar methods were employed, but a thin layer of glass wool about half-an-inch above each substance prevented the animals from touching the latter. Further, the tubes were plugged with moist cotton-wool and placed in a hot-house.

In the case of (*c*), 250 grammes of fresh soil were thoroughly mixed with one-hundredth part of the molecular weight of each substance in grammes, the mixture being placed in a glass jar 8 inches high and 3 inches in diameter. Ten woodlice were placed on the surface of the soil, more being added at required intervals.

Controls were set up in each case, as these woodlice are liable to be adversely influenced by lack of food over long periods of time. Into some of the soil jars, 20 c.c. of water were added from time to time to prevent any serious desiccation.

In the controls whether water was added to the soil at the beginning of the experiment or not, the woodlice were all living 29 days after being placed in the jars, all being dead on the 36th day, but if separated from the soil, by a piece of wire gauze, they began to die after only four days, and none survived six days. Again, if water was added to the soil after being 29 days in a jar, and fresh woodlice were introduced, they seldom survived more than four days. This all points to exhaustion of food in the soil, and not to lack of moisture, as being the cause of death.

It is readily admitted that in some cases, death may have resulted from starvation through contamination of the food-supply by a compound used, though in only one case (Phenanthrene) could death have been attributed to actual poisoning through the intestine. This would account for the comparatively large number of deaths in soil from which a substance has presumably almost disappeared.

Action by Contact.

The total duration of these experiments was 17 hours. At the end of this period, one out of the five woodlice used was dead in the control. When any of the woodlice in the tubes were still living at the end of the experiment, the substance employed was deemed to have no action.

In the following tables the maximum and minimum times in which death and paralysis are brought about by the various compounds are given:

Contact Action. Temperature 64–72° F.

Death in minutes	Paralysis in minutes	Substances
1– 2	1	Phenol, *o*-Cresol, *p*-Cresol
6– 36	2– 6	*p*-Nitrophenol, Camphene
33– 62	31– 41	*p*-Nitrophenol
60– 75	16– 38	Thymol, Camphor
75– 90	20– 70	Pyrogallol, Resorcinol
90–150	24– 60	Pyrocatechol, *α*-Naphthol, Naphthalene
150 upwards	40–100	Hydroquinone, *β*-Naphthol, Menthol

In the case of Phenanthrene, there was a slight contact action, two woodlice out of five still showing signs of movement at the end of 17 hours. Pyrocatechol, *α*-Naphthol and Hydroquinone tended to darken the gill-plates. The Nitrophenol stained the appendages and carapace yellow. Anthracene, Anthraquinone, Alizarin, Phloroglucinol and Borneol were without action.

Action by Vapour.

The duration of the experiments was limited to 21 hours. In the controls, which consisted of one tube plugged with dry and another with wet cotton wool, the woodlice were all alive at the end of this time.

Vapour Action.

Death in minutes	Paralysis in minutes	Temperature ° F.	Substances
5 – 17	1½–12	70– 96	*o*-Cresol
24 – 34	3½–19	70– 98	Phenol
27 – 50	7 –30	70–100	*p*-Cresol
50 –100	23 –75	70– 90	Camphor, Camphene, *o*-Nitrophenol, Thymol
150 upwards, Menthol, *α*-Naphthylamine, Naphthalene, Anthracene, Anthraquinone			
Also tested			
8 – 50	2 –14	69– 90	*o*-Dichlorbenzene
12 – 90	6 –42	69– 90	*p*-Dichlorbenzene
9¼– 17	6½–13½	86	Pyridine

The following were without action: *p*-Nitrophenol, Hydroquinone, Resorcinol, Pyrocatechol, Pyrogallol, Phloroglucinol, *α*-Naphthol, *β*-Naphthol, Alizarin, Borneol. *o*-Nitrophenol stained the woodlice yellow. The appendages were much blackened by Anthraquinone. The inactivity of Hydroquinone shows that this substance acts by contact upon the

gill-plates of the animals, causing death by affecting the external respiratory system.

Action in the Soil.

The controls have already been referred to, and curves given of the quicker acting compounds. All the compounds were carefully mixed with 250 grammes soil in the following proportions, and the experiment carried out at room temperature, except when otherwise stated:

Phenol 1 part in 265 parts soil.
o-Cresol, *p*-Cresol, 1 part in 231 parts soil.
o-Nitrophenol, *p*-Nitrophenol, 1 part in 108 parts soil.
Resorcinol, Pyrocatechol, Hydroquinone, 1 part in 227 soil.
Pyrogallol 1 part in 198 soil.
Phloroglucinol 1 part in 154 soil.
Naphthalene 1 part in 195 soil.
α- and β-Naphthol 1 part in 173 soil.
α-Naphthylamine 1 part in 174 soil.
Anthracene, Phenanthrene, 1 part in 140 soil.
Alizarin 1 part in 104 soil.
Anthraquinone 1 part in 120 soil.

Camphene	1	,,	183	,,
Camphor	1	,,	164	,,
Menthol	1	,,	160	,,
Borneol	1	,,	162	,,
Thymol	1	,,	166	,,

Resorcinol, Pyrocatechol, Pyrogallol, Phloroglucinol, β-Naphthol, Anthracene, Alizarin, Anthraquinone, had no action; Borneol killed 30 per cent. during the first five days, Camphene 40 per cent. during the first three days, the action ceasing in each case at the period mentioned.

Phenol. 100 per cent. woodlice killed within 24 hours up to the 52nd day after mixing. (On the 44th day after mixing paralysis set in $1\frac{1}{4}$ hours after the woodlice were put in the jar.)

Concentration 1 part in 500 soil (25 c.c. of a 2 per cent. solution in water). 100 per cent. woodlice killed within 24 hours up to the ninth day after mixing. Action ceases on the 14th day after mixing.

Concentration 1 part in 750 soil (20 c.c. of a 1·65 per cent. solution in water). 100 per cent. woodlice killed within 24 hours up to the sixth day after mixing. Action ceases on the ninth day after mixing.

Concentration 1 part in 1000 (25 c.c. of a 1 per cent. solution in water). 80 per cent. woodlice killed in the first 24 hours after mixing, then

100 per cent. killed in the second 24 hours after mixing. Action ceases on the fourth day after mixing.

A concentration of 1 part Phenol in 2000 parts soil had no action.

o-*Cresol.* 100 per cent. woodlice killed within 24 hours up to the 25th day after mixing. Action ceases on the 38th day after mixing.

p-*Cresol.* 100 per cent. woodlice killed within 24 hours up to the 21st day after mixing. Action ceases on the 32nd day after mixing.

o-*Nitrophenol.* 100 per cent. woodlice killed within 24 hours up to the 26th day after mixing. Action ceases on the 49th day after mixing. The appendages and gill-plates are stained yellow.

p-*Nitrophenol.* Slow action. 100 per cent. killed in 10 days up to the 20th day after mixing. Maximum activity from 20–23 days after mixing. Action ceases 55 days after mixing. The appendages and gill-plates are stained yellow.

Hydroquinone. 100 per cent. killed in three days up to the fifth day after mixing. Maximum activity from sixth to eighth day after mixing. Action ceases on the tenth day after mixing. In solution in water at equivalent strength the maximum activity takes place from fifth to seventh day and action ceases on the eighth day after mixing. At hot-house temperature, the maximum activity was shown from fourth to sixth day, action ceasing on the seventh day after mixing.

A characteristic attitude is taken up by the woodlice when placed in the jars; they remain on the surface of the soil, and after 24 hours they become inactive, the segments of the thorax and abdomen are extended, but compressed from side to side. The thoracic appendages move weakly, and the outer division of the tail appendages are held upwards and outwards. The gill-plates of the abdomen become dusky, and turn brown shortly before death. It has been observed that the dark colour extends into the tracheal tubes connected with the gill-plates. It is concluded that Hydroquinone enters the system through actual contact with the abdominal appendages and passes from them into the respiratory system. Deaths in the jars from the eighth to seventeenth day after mixing indicate lack of nutriment.

Naphthalene. 100 per cent. woodlice are killed in the first 24 hours after mixing. The action then becomes slightly slower and ceases on the fourth day. It is probable that the nerve-endings on the surface of the animal are affected: paralysis sets in early and is of considerable duration before movement ceases.

α-Naphthol. Action starts within the first 24 hours, and is at its maximum till the fifth day after mixing. Whereas most are killed during the first two days, some will survive for eight days. From the second to fourth day, the action is more definite, but later the woodlice die irregu-

larly, some surviving for at least 11 days after being placed in the jars. The action appears to cease on the ninth day after mixing. Similar results were obtained at hot-house temperature.

α-Naphthylamine. 100 per cent. woodlice are killed within 24 hours up to the 28th day after mixing. The rate of action is then about halved till the 64th day, and after the 66th day from mixing action ceases. The period of paralysis before death is extremely short, and it is possible that death is produced by vapours entering the respiratory system.

Phenanthrene. Experiments at hot-house temperature. The action is slow and very irregular. From the large number of deaths from the ninth day after mixing onwards, it is probable that the woodlice eat this compound when the food supply becomes scanty. No definite paralysis preceded death, the movement simply becoming feeble and the segments of the animals contracted.

Camphor. Experiment at hot-house temperature. The action is weak, 90 per cent. being killed from the first to fifth day after mixing, with a maximum activity (100 per cent. in three days) from the third to fifth day; after the sixth day, there appeared to be no action. Some woodlice were entirely unaffected from the start of the experiment, and seemed more resistant than others.

Menthol. Experiment at hot-house temperature. The action is at first slow, and gradually increases to the ninth day after mixing. It was not determined how long the activity lasted.

Thymol. Experiment at hot-house temperature. The action is at first slow, but after the third day from mixing, 100 per cent. woodlice are killed within 48 hours until the ninth day. Some woodlice are much more resistant than others when placed in the soil at the start of the experiment, but later the action becomes definite.

SUMMARY.

With a view to the winter control of the cucumber woodlouse the action of a number of compounds has been tested. Observations are described on the effects due to contact, vapour and mixing with the soil.

The organism of the animal is not affected in a similar manner by different compounds. *p*-Cresol and *p*-Nitrophenol are less active than the corresponding ortho-compounds and both nitro-phenols are less active than phenol.

Certain compounds show a maximum activity shortly before their disappearance from the soil. Phenol and the cresols were the most active compounds tested. Naphthalene ceases to act in the soil on the fourth day after mixing.

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A ROOT DISEASE OF THE TOMATO CAUSED BY *COLLETOTRICHUM TABIFICUM* (HALLIER *PRO PARTE*) PETHYBRIDGE

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and J. SHEARN.

(With Plates V and VI.)

During an investigation of tomato root-rots in 1919, cases of a distinctive type were met with in which very numerous, minute, black sclerotia were found embedded deeply in the woody tissues. The causal organism was isolated, its pathogenicity proved and cultures were set aside for further study when circumstances permitted. In 1920 a culture of the same fungus, which he had isolated from a diseased tomato root, was sent to us by Dr H. Wormald. In 1921 the disease assumed increased importance and in the Lea Valley it was continually found during the summer. When roots were examined at the end of the season, many hundreds were discovered in an advanced state of decay, and containing the sclerotia. Further information concerning the disease was obtained in 1922, when a systematic inspection of commercial nurseries indicated that it was of considerable economic importance in nurseries where tomatoes had been grown continuously for several years.

During the season of 1923 the disease appeared unusually early and cases of death from this cause were observed in May and June. At this time it was apparent that the constitution of the tomato crop had been appreciably weakened by the unfavourable weather conditions which had prevailed. This became accentuated later by the absence of sufficient sunlight to ripen the fruit quickly and permit of its being gathered, and the heavy trusses of green fruit thus remaining caused a severe drain on the plants.

SYMPTOMS OF THE DISEASE.

The symptoms of the disease do not differ in any material respect from those usually exhibited by tomato plants, the roots of which are being slowly destroyed by other parasitic fungi. Such plants cease to

develop in a normal manner; the lowest leaves turn yellow and die prematurely, while any new growth is weak and pale in colour. In advanced stages, the stem becomes yellow and hollow, while a dark brown discoloration sometimes appears on the outside of the stem near the ground and spreads rapidly up the plant. Usually the discoloured streak is about a quarter of an inch in width and occurs on one side of the stem only. On pulling up a diseased plant, the roots offer no resistance, as they are dry and shrivelled; and the decayed base of the stem separates readily from the soil. If the roots are carefully dug out, they are seen to be completely decayed and brown. The tissues external to the wood are destroyed and shrivelled, and fit quite loosely over the central woody cylinder. The distinctive feature is the presence of numerous minute, black sclerotia both external to and embedded in the wood (Pl. V, fig. 1). These are most commonly confined to the roots, but in bad cases the base of the stem below ground and even its lower aerial part may contain sclerotia. The infected wood varies from "Light Brown[1]" in the root to "Wood Brown" in the stem, and the wood of the stem may be discoloured up to a distance of two and a half feet from soil level. The colour of the darkest part of the discoloured stem wood is "Saccardo's Amber" in cross section and "Wood Brown" in longitudinal section, while, not infrequently, a beautiful amethystine colour pervades the wood. Sclerotia are most numerous in the roots but they have been found in the stem wood as far as 28 inches above the soil. When situated on the outside of the wood or in pith cavities, the sclerotia are liberally provided with stiff black hairs or setae, which are absent when these bodies are formed within the wood elements.

MORBID ANATOMY.

Infection takes place through the roots, and a wound does not appear to be necessary. The finer roots are first attacked, and the fungal hyphae spread rapidly through the tissues up to the stem base and finally may pass into the aerial portions of the stem, where they have been found up to 30 inches above ground level.

Investigation has shown that the root cortex is first attacked, the rapid destruction of the cells leaving only a hard dry envelope lying loosely round the central woody core. The invading hyphae readily enter the woody tissues, and, although not confined to any particular type of element they are generally most abundant in the larger vessels. Except in the immediate vicinity of the sclerotia, the hyphae are hyaline, but

[1] Ridgway, *Color Standards and Nomenclature.*

their component cells are frequently swollen and irregular in shape indicating a tendency to produce sclerotia. These latter bodies are produced mainly when the hyphae reach the larger vessels or the exterior and interior of the woody cylinder where the cortex or the pith respectively has been destroyed. A sclerotium within a vessel gradually increases in size until the lumen is completely filled, and in such situations setae are rarely produced. The hyphae on the outside of the sclerotium do not in this case form a dense dark layer but continue to develop, and, by passing through the pits in the vessel-walls, grow into the surrounding elements. Thus a large sclerotium may in time extend over a considerable number of wood elements. Whilst the tendency of development is to follow the line of least resistance by spreading up and down the vessels, so that longitudinal sections show the sclerotia as being long and narrow, considerable spreading also takes place transversely. As a general rule the walls of the xylem elements surrounded by sclerotial masses show no sign of undergoing change by reason of fungal secretion, but occasionally the walls are seen to be blackened and are apparently brittle.

Sclerotia formed on the outside of the wood or within the pith cavity are more regular in shape than those produced internally, and are usually provided with setae.

Comparative observations have shown that the wood of tomato plants infected by this fungus contains a considerably greater amount of mycelium than that of plants attacked by either *Fusarium lycopersici* or *Verticillium albo-atrum.*

INOCULATION EXPERIMENTS.

No difficulty was experienced in isolating the causal organism, for it readily grows out from small pieces of diseased wood removed aseptically and placed in the centre of poured potato-agar plates. Typical sclerotia are produced on this medium in five or six days. The organism grows well on artificial media and on sterilised vegetable tissue (Pl. V, fig. 2). Its pathogenicity was readily proved by growing healthy plants in sterilised soil copiously contaminated with sclerotia obtained from a pure culture. The lower leaves of such plants became yellow and drooped within six weeks of planting, while those of suitable controls remained green and turgid. Within three months of planting, ten plants out of twelve were stunted in comparison with the healthy controls. At this time the roots of all twelve plants were in an advanced state of decay, showing typical sclerotia, and the parasite was readily re-isolated. The roots of the control plants were perfectly healthy and showed no signs

of sclerotia, while all attempts to isolate a parasitic organism from them failed.

Attempts were made to infect healthy tomato plants (eight weeks old) by inoculating the aerial portions with pieces of mycelium from a pure culture, but with the exception of a browning of the tissues for a distance of about 1 cm. round the inoculation stab, and the consequent production of sclerotia within this restricted area, no positive results were obtained. On the other hand, infection was readily produced by stab inoculations on healthy roots, when the stabs were covered with tin foil and the soil replaced.

Tomato fruits both green and red are quickly rotted by the fungus, if the latter is introduced through a wound. In green fruits a soft brown area is produced round the inoculation point by the second day. This area rapidly spreads, and by seven days after inoculation it may extend half over the fruit. The hyphae ramify through the soft flesh forming an intricate network on which sclerotia are produced freely. The hyphae also break through the skin producing numerous sclerotia on the outside. Natural infection of the fruit has not been observed.

In view of these results it would appear that the disease is contracted in the soil, and that primary infection of the aerial portions rarely, if ever, occurs.

THE CULTURAL CHARACTERS AND IDENTITY OF THE CAUSAL ORGANISM.

In a previous note (1) the fungus causing this disease was described as a new species, *Sclerotium setosum* Bewley and Shearn. Some months after publication a typical *Colletotrichum* fructification was observed on an old culture on cellulose agar, and this led to further investigation.

The characters of the fungus in question recall the published descriptions of Ducomet and others (4, 5, 7) relating to a disease of potatoes and tomatoes due to *Vermicularia varians* Ducomet, and O'Gara's description (8) of *Colletotrichum solanicolum*. They also resemble those of *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge, described by the latter as a weak parasite on the potato in Ireland; but the consistent absence of any organised condial fructification during three years' cultivation in the laboratory seemed to distinguish it as a separate entity. Pethybridge (9) in describing his investigations concluded that his fungus was identical with that described by Hallier (6) as *Rhizoctonia tabifica*, and noted its similarity to *Vermicularia varians* Ducomet.

The tomato fungus grows readily upon a wide range of media, and for laboratory work, Dox's agar with 1 per cent. saccharose has proved satisfactory. Growth on this medium is rapid and the mycelium develops as a white compact layer over the surface of the medium. Within 14 days of inoculating the medium, the fungal growth assumes a "Shrimp Pink" colour, which remains for 7 to 10 days and then disappears, leaving innumerable minute "Shrimp Pink" masses about 200μ in diameter. These masses slowly increase in size, lose their pink colour and turn black.

The mycelium consists of branched septate hyphae, generally well filled with granular cell contents, which stain deeply with neutral red, and oil drops are plainly visible. Swollen cells, round, oval and pear-shaped are present in the mycelium, and vary in quantity in accordance with the medium. Thus upon inulin agar, casein agar, ethyl butyrate agar and Dox's agar with 1 per cent. saccharose, but few swollen cells are found, while on amygdalin agar, blood fibrin agar, and tyrosin agar they are abundant.

Conidia are produced either on conidiophores or in acervuli. The conidiophores bearing sessile conidia appear in young cultures some 24 hours after seeding with spores or sclerotia, and are also found at the edges of rapidly growing cultures, or at the tops of sloped tube cultures. This is the commonest form of spore production, but occasionally in old cultures both *Gloeosporium* and *Colletotrichum* fructifications may be found, the former direct on the medium, the latter on the surface of the sclerotia. The *Colletotrichum* acervuli appear as watery, dark or amethystine coloured globules surmounting old setae-bearing sclerotia usually at the bottom of a tube slope. Frequently, in hanging drop cultures of germinating spores, appressoria are produced, but perhaps the most characteristic feature of this fungus is the rapid production (usually in 7 to 10 days) of minute black sclerotia on most media. These are similar in appearance to those found on the plant, but whereas here (at least when they are on the exterior and not embedded in the wood) they are liberally provided with dark brown setae, on most media setae are absent. The number of sclerotia produced on each square centimetre of the plate, and the modal size of the sclerotia vary with the kind of medium, as shown in Table I.

The appearance of the *Colletotrichum* fructifications previously mentioned led to further examination of the tomato fungus in parallel with *C. tabificum* and *V. varians*, cultures of which were kindly supplied by Dr G. H. Pethybridge.

Table I.

Medium	Average no. of sclerotia per sq. cm.	Modal size of sclerotia in microns	Percentage of small sclerotia $90 \times 100\mu$ in size
Aesculin agar	19	230 × 240	20
Amygdalin agar	159	250 × 300	Centre of Plate 0, edge 80
Arbutin agar	None	None	None
Blood fibrin agar	26	230 × 240	3
Casein agar	35	240 × 250	40
Cellulose agar	31	360 × 450	5
Dox's agar with 1 % saccharose	94	520 × 570	10
Ethyl butyrate agar	55	150 × 170	60
Inulin agar	72	330 × 420	15
Starch agar	39	300 × 400	12

Single spore cultures of the three fungi were grown on seven different media. Plates containing a uniform thickness of medium were prepared in triplicate, and inoculated by placing a sclerotium from a single spore culture in the centre of each plate. After 56 days' incubation at laboratory temperature, six counts of the numbers of sclerotia were made in each plate, and the average of three plates recorded. The results are given in Table II.

Table II.

Showing number of sclerotia per sq. cm. of medium after 56 days.

Medium	*Colletotrichum tabificum*	*Sclerotium setosum*	*Vermicularia varians*
Aesculin agar	25	19	26
Amygdalin agar	185	156	124
Arbutin agar	None	None	None
Casein agar	47	35	72
Ethyl butyrate agar	25	34	46
Inulin agar	91	72	85
Starch agar	52	39	48

The amethyst coloration emphasised by Pethybridge (9) appears irregularly in cultures from all three sources, but does not seem to be a constant feature. These results indicate a marked degree of similarity between the three fungi (Pl. VI). They all produce sclerotia and conidia both on conidiophores and in acervuli. Dickson (3) in 1923 described the occurrence of a saltation in cultures of the organism causing "Black Dot" (*Vermicularia varians*) disease of potato tubers in Canada. This is of interest in view of the fact that cultures of the three organisms on Dox's agar with 1 per cent. saccharose have all shown a marked tendency to this phenomenon.

In a recent paper (2) Cavadas, working with *V. varians*, reports the

discovery of specialised conidiophores bearing sessile conidia. From his description and figures it is apparent that these are the same bodies as are found in cultures of the tomato fungus. He records further the development of *Colletotrichum* acervuli on media rich in readily assimilable salts, but was unable to find *Vermicularia* structures. This is in complete accord with our own experiences.

Further evidence of identity was sought in inoculation experiments. Sterilised soil was contaminated by the liberal incorporation of steamed tomato-stem cultures of the fungi in sets of twelve, an equal number of non-contaminated controls being left. Six tomatoes and six potatoes were planted in each set of twelve pots. The roots were removed two months later, and obvious infection was found in those from the soil contaminated with all three fungi, while those from the uncontaminated pots were perfectly white and healthy. These results (Table III) coupled with those obtained by the cultivation of the three fungi in various media lead to the conclusion that they are identical.

Table III.

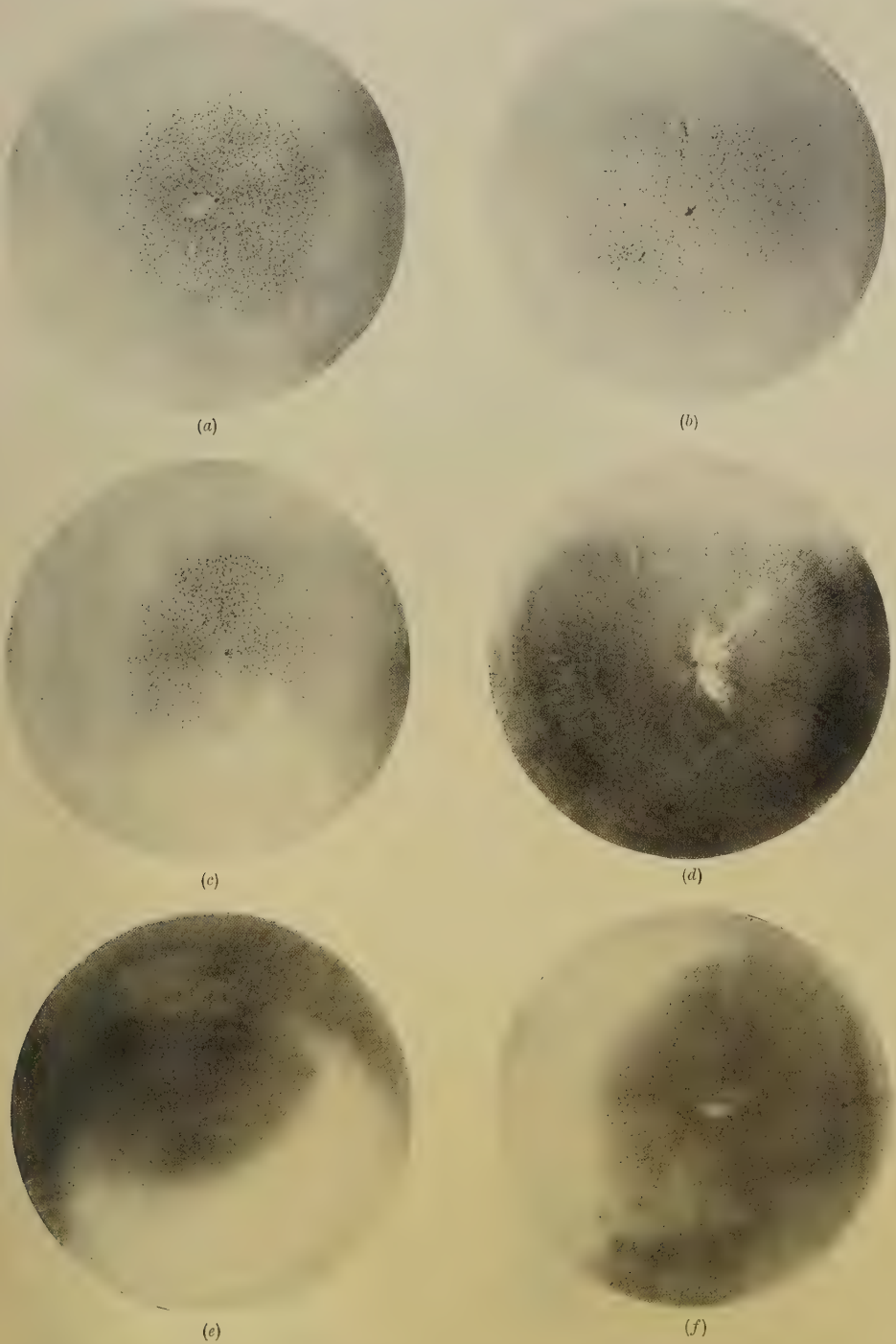
Plant	No. used	Date planted in contaminated soil	Date examined	Fungus employed	No. of plants infected	No. of plants healthy
Tomato	6	2. x. 23	28. xi. 23	Control	—	6
„	6	„	„	*C. tabificum*	5	1
„	6	„	„	*S. setosum*	6	0
„	6	„	„	*V. varians*	5	1
Potato	6	„	„	Control	—	6
„	6	„	„	*C. tabificum*	4	2
„	6	„	„	*S. setosum*	5	1
„	6	„	„	*V. varians*	4	2

The question of nomenclature still remains. In view of the fact that no observer has been able to repeat Ducomet's findings of *Vermicularia* fructifications, and that *Colletotrichum* acervuli are constantly obtained, the placing of the fungus in the latter genus seems justified. It is possible that O'Gara's *C. solanicolum* is identical with the fungus now being dealt with, but the absence of inoculation trials leaves this question in doubt. At the same time examination of Hallier's description and figures (6) illustrating *Rhizoctonia tabifica* leaves little doubt that Pethybridge was correct in assuming Hallier's fungus to be identical with that which he isolated from the potato. It is considered therefore that *Colletotrichum tabificum*, *Sclerotium setosum* and *Vermicularia varians* are identical with *Rhizoctonia tabifica* and that the name *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge should stand.

Fig. 1

Fig. 2

BEWLEY & SHEARN.—A Root Disease of the Tomato caused by *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge. (pp. 244—251.)



(*a*) (*b*) (*c*) (*d*) (*e*) (*f*)

BEWLEY & SHEARN.—A Root Disease of the Tomato caused by *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge. (pp. 244—251.)

CONTROL OF THE DISEASE.

At the present stage little can be said about the control of the disease in question, but further investigations are in progress, the results of which will be given later.

SUMMARY.

1. A root disease of the tomato, due to *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge, has been investigated and described.

2. The pathogen is considered to be identical with *Rhizoctonia tabifica* Hallier, *Vermicularia varians* Ducomet, *Sclerotium setosum* Bewley and Shearn, and possibly *Colletotrichum solanicolum* O'Gara.

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EXPLANATION OF PLATES V AND VI

Plate V.

Fig. 1. Lower portion of diseased tomato stem showing the minute black sclerotia of *Colletotrichum tabificum.*

Fig. 2. Cultures of *C. tabificum* on wheat straw and Dox's agar.

Plate VI.

(*a*) *C. tabificum* on starch agar.
(*b*) *S. setosum* ,,
(*c*) *V. varians* ,,
(*d*) *C. tabificum* on Dox's agar.
(*e*) *S. setosum* ,,
(*f*) *V. varians* ,,

(*Received March 5th,* 1924.)

INVESTIGATION OF THE STRUCTURE OF REVERTED BLACK CURRANTS

By WINIFRED F. F. RIDLER, M.Sc.

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(With 4 Text-figures and Plate VII.)

Lees (2) described a method for identifying cases of Reversion in Black Currant plants by means of certain morphological characteristics, namely, the number of sub-main veins on the leaf, and the nature of the leaf margin; the number of sub-main veins running from the midrib to points in the margin; and the number of margin points being numerically fewer in reverted leaves than in normal ones.

In examining black currant plants Lees found that there were three main classes: normal healthy plants, "simple reverted" where there is apparently no mite infection, but where mite infection has probably been at work, and the plant is still suffering from the effect of some previous infection; and "mite infection (reverted)" where mite is still present. Corresponding with these, three factors appear to be at work: a seasonal factor tending to reversion of a temporary character; a reversion factor; and a mite factor; and it is usually extremely difficult to separate the two last, and to be quite sure whether fall in leaf vein number is due to the reversion factor only and not the mite factor, or whether it may be due to both.

The object of the present investigation, which was undertaken at the suggestion of Mr Lees, was to ascertain if there were any anatomical differences between reverted and normal plants, and if so, whether such differences could be correlated with the morphological evidences of the disease.

For this purpose a large number of normal and reverted plants were examined, and sections have been cut from every part of the plant. All the material used was obtained from the Agricultural and Horticultural Research Station, Long Ashton.

ROOT.

A very young root of the normal plant, before secondary thickening has begun, has not been examined, but a root with one ring of secondary growth shows clearly the primary wood in the centre, usually either triarch or tetrarch, with the smaller protoxylem elements towards the outside, and intermixed with parenchyma. The secondary wood consists of vessels and tracheids. In older roots the annual rings are very clearly marked. Aggregate medullary rays occur opposite the protoxylem elements. The phloem consists of multiseriate zones of soft bast without crystals, alternating with usually uniseriate rows containing crystals of calcium oxalate, the number of the latter increasing with the age of the root. There is a wide zone of phelloderm, and a narrow cork zone.

There is apparently very little difference between the normal and reverted roots. There is less parenchyma amongst the primary wood of the revert, and in the secondary wood, the vessels and tracheids are slightly smaller than in the normal, measuring 5–45 μ in diameter as compared with 7–50 μ in diameter in the normal. The medullary rays are wider in the reverted root measuring 7–28 μ in width, as compared with 7–18 μ in the normal; on the whole, therefore, the area of the medullary ray tissue in comparison with that of the wood is larger than in the normal, though this is not so definite as in the petiole and leaf (see below).

STEM.

In the young normal stem before secondary thickening takes place, there is a relatively large pith which is heterogeneous in character consisting of large and small-celled parenchyma. The primary xylem consists of rows of spiral and annular vessels measuring 18–36 μ in diameter, between which are medullary rays one or two cells in width. The cambium is well marked, and the phloem consists of radial rows of large parenchymatous cells with no contents, alternating with small cells with dense contents and well-marked nuclei. Outside the pericycle and endodermis is the cortex composed of large parenchymatous cells, and two layers of epidermal cells.

In the old stem, cork formation takes place in the pericycle, and the bundles consist of wood, consisting of isolated vessels with scalariform thickening, and wood fibres which are prosenchymatous. The bast as in the root, consists of multiseriate zones of soft bast without crystals, alternating with usually uniseriate uninterrupted zones containing clustered crystals (De Bary (1)).

There is very little difference between the normal and reverted stem. The cuticle of the epidermal cells is somewhat thicker in the revert than in the normal. The wood cells are somewhat smaller in the revert, measuring 14·5–30 μ in diameter as compared with 18–36 μ in the normal, and have rather less definite contours. There are apparently no differences in the secondary tissues.

PETIOLE.

Three vascular strands pass into the petiole, and at the stem end these consist of wood vessels and prosenchyma, with medullary rays, usually uniseriate in character, and with dense contents. The phloem, as in the young stem, consists of radial rows of large parenchymatous cells which here are full of a dense gummy substance which may stain blue, red or yellow with haematoxylin and safranin; and small cells with protoplasmic contents and thinner walls. At the outer limit the phloem consists almost entirely of these large cells full of the dark staining substance. Further up towards the leaf, the walls of these cells are thickened, forming a zone of sclerenchyma. At the leaf end the sclerenchymatous zone is once more absent. The ground tissue consists of large thin-walled parenchymatous cells, somewhat heterogeneous in character.

The differences between the normal and reverted petiole are much more marked than in the stem (Figs. 1 and 2).

In the ground tissue and phloem the structure is the same but there is rather more of the red and yellow staining substances present in the revert and less of the blue. The medullary rays in the reverted petiole are usually two cells in breadth instead of one as in the normal; the cells are considerably larger, measuring 7–10 μ in diameter as compared with 3·5–9 μ in the normal. Their area compared with that of the wood is therefore larger in the reverted petiole than in the normal. They are less regularly arranged in the revert, and the cells like those of the phloem contain more of the red and yellow gummy substance.

The xylem elements in the reverted petiole, as might be expected from the condition of the medullary rays are also less regularly arranged. The vessels in the reverted petiole have less definite contours, and are considerably smaller than in the normal, measuring 15–20 μ in diameter, while in the normal they are 20–25 μ in diameter; the area of the wood as compared with that of the medullary rays is consequently smaller. The walls of the vessels are often thickened and stain bright red with safranin instead of the normal dull red; when this occurs, the middle lamella

Fig. 1. Portion of bundle of petiole of a normal black currant. ×250.

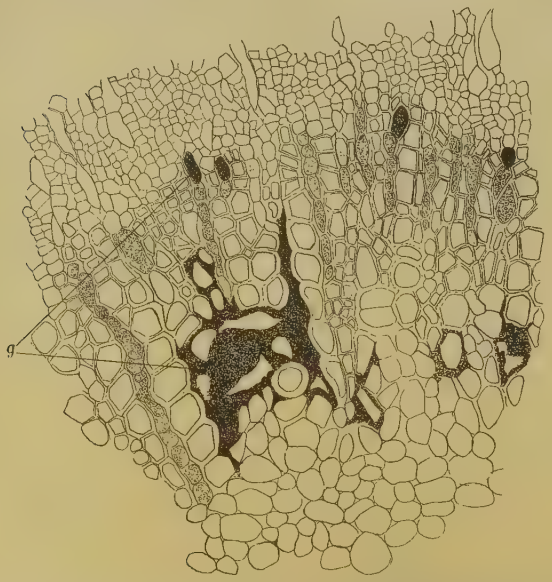

Fig. 2. Portion of bundle of petiole of a reverted black currant, showing irregularity of wood and accumulation of gum. ×250. *g* = gummy substance.

becomes very indistinct or may not be visible. A gummy substance which stains bright red with safranin, similar to that in the phloem, may be produced, completely blocking some of the cells or entirely destroying them. This resembles somewhat the formation of wound gum, but the substance produced in *Ribes* gives none of the reaction for wound gum. The substance occurs normally in all parts of the plant, and is probably similar to, or the same as, that excreted from the glands on the leaves and flowers. It is insoluble in water, alcohol and xylol; sulphuric acid and caustic potash apparently have no effect upon it, and it does not stain red with phloroglucin and hydrochloric acid. Apparently it tends to be produced in greater quantities in reverted than in normal plants.

LEAF.

The leaf of *Ribes* has a bifacial structure; the upper epidermis consists of large cells somewhat irregular in size, and the lower of smaller cells more regular in shape and size, and with stomata with small guard cells (Solereder (3)). The palisade tissue consists of two layers of cells. Below the bundles at the main and sub-main veins is a large-celled parenchyma; clustered crystals occur in some of the cells of this tissue, and also in certain cells of the palisade and spongy parenchyma.

The phloem of the bundle consists as in the petiole, at the outer limit of large parenchymatous cells full of the, here, blue-staining substance; and further in of radial rows of these large cells alternating with the smaller cells with protoplasmic contents. No sclerenchyma is developed in the phloem. The cambium zone is well marked, and the wood consists of vessels regularly arranged, alternating with medullary rays.

The differences between the normal and reverted leaves are, as might be expected, of the same order as those in the petiole but on the whole less well marked.

There is apparently no difference in the phloem, but the wood vessels in the normal are more regularly arranged, and have more definite contours than in the revert; moreover, they are larger in the normal, measuring 15–45 μ in diameter (in the main vein) while in the revert they measure 12–35 μ in diameter. There is on the whole less wood in the revert than in the normal. The area of the medullary ray tissue is correspondingly larger in the revert, the width of the ray being 7·5–15 μ as compared with 5–12 μ in the normal (Figs. 3 and 4).

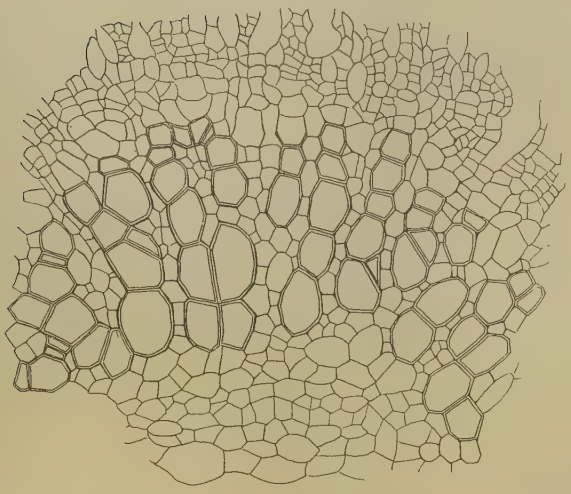

Fig. 3. Portion of bundle of leaf of a normal black currant. ×250.

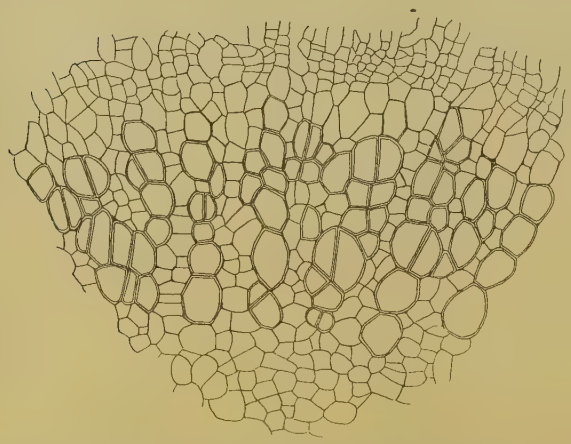

Fig. 4. Portion of bundle of leaf of a reverted black currant, showing irregularity of wood, smaller vessels and wider medullary rays. ×250.

PEDUNCLE AND PEDICEL.

The peduncle and pedicel have essentially the same structure as the young stem. The phloem has the same typical structure as in the stem petiole and leaf; the cambium zone is well defined, and the xylem consists of rows of vessels alternating with medullary rays, though these are not as definite as in the stem owing to the smaller size, and consequent reduction in the relative amounts of the various tissues.

Here again the differences between the normal and reverted petiole are of the same order as have been noted in the rest of the plant. The phloem in the reverted peduncle has considerably more of the red staining substance and less of the blue (cf. Petiole). The medullary ray tissue is larger in comparison with the wood, and the cells as in the phloem contain more red staining substance. In the xylem the cells are distinctly smaller in the revert, measuring 3·5–10·5 μ in diameter, while in the normal they are 3·5–18·0 μ in diameter. The area in comparison with the medullary ray tissue is smaller than in the normal, and the vessels are less regularly arranged.

FLOWER AND FRUIT.

The fruits of black currant plants suffering from reversion either do not form at all, the flowers themselves shrivelling up and dropping off; or the fruits may begin to form, in which case development is soon arrested and the half-formed berries shrivel and fall.

Sections through a normal unripe fruit show a large well-developed, and profusely branched vascular system. The wood vessels are large and numerous, and there is a considerable quantity of the blue and red staining substances in the phloem, and cortex surrounding the bundle. The ground tissue consists of large parenchymatous cells.

The ovary is large and well-developed and the ovules are normal. There is a layer of large cells containing the blue staining substance around each ovule; around the embryo-sac there is another layer of smaller cells containing either the blue, red or yellow staining substances; this layer widens into a cushion of cells at the antipodal end also containing the red or yellow staining substances. The nucellus in the earlier stages is well marked and healthy, and reproductive nuclei are visible in some cases (Pl. VII, fig. 5).

In the reverted plant, where formation of the fruit has begun, the condition of the fruit as shown by sections is very different. The vascular system is reduced and not so profusely branched; the wood

vessels are smaller in size and fewer in number, and there is considerably less of the blue staining substance in the phloem.

The ovary is small and poorly developed, and the ovules reduced and hypertrophied. The layer of large cells surrounding each ovule is absent except in one or two cases—this is very striking. The nucellus in most cases is unhealthy and the cells are degenerating; no embryo-sac is developed and no reproductive nuclei (Pl. VII, fig. 6).

In some cases where the fruit has not begun to develop and the whole flower has dropped off, sections show that the vascular system is still more reduced and may even be absent altogether. An enormous amount of gummy substance may be produced at the base of the ovary, and in some cases completely filling all the ground tissue; what vascular system is present being blocked by this. The ovary is very reduced in size, and the ovules are aborted. The stamens are hypertrophied and the pollen grains arrested in development (Pl. VII, fig. 7).

GROWING POINT.

Sections were cut through the growing points of normal stems, reverted stems without big buds, and reverted stems with big buds.

No difference is apparent between the first two. In the third, however, as would be expected, the growing point is flattened, growth being in a lateral direction rather than a vertical, thus giving rise to a big bud.

GENERAL CONCLUSIONS AND SUMMARY.

No actual qualitative differences in structure have been observed between normal and reverted plants of *Ribes nigrum*; those that have been observed being rather quantitative, *i.e.* differences in degree only, *e.g.* amount of gum present, and variations in the amount of wood and medullary ray tissue.

Briefly the main differences showing to a greater or lesser extent practically all through the plant are a reduction in the amount of wood, and a consequent increase of medullary ray tissue, coupled with a tendency to produce more gum in the revert plant than in the normal.

On the whole, it is very difficult to correlate the morphological evidences of the disease with any of the anatomical differences observed, as the latter seem quite inadequate to account for the comparatively large morphological effect.

The general reduction in the amount of wood present, does not seem sufficient to account for the generally smaller and more unhealthy appearance of the revert bush; nor in the petiole even combined with

a certain amount of blocking by gum, can it be held to account entirely for the typical reverted leaf, with fewer veins and reduced leaf surface. In the case of the fruit, although the vascular system is reduced, there seems no reason why some of the ovules at least should not develop. It is probable that there is some physiological factor at work causing hypertrophy and abortion.

My thanks are due to Mr A. H. Lees, who has supplied me with all material and given much valuable help and advice. I also thank Mr F. Summers and Professor O. V. Darbishire for their advice and interest in the work.

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EXPLANATION OF PLATE VII

Fig. 5. Transverse section of ovary of normal black currant. ×25.

Fig. 6. Transverse section of ovary of reverted black currant. ×25.

Fig. 7. Longitudinal section through flower of reverted black currant showing complete blockage by gum. ×25.

(*Received January 20th*, 1924.)

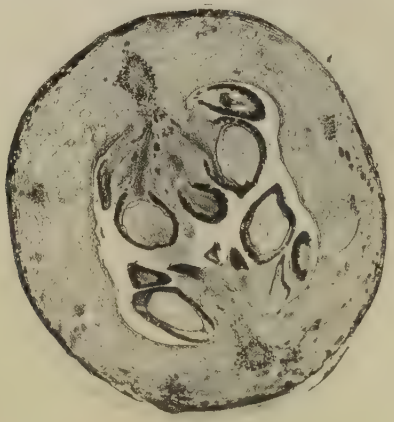

Fig. 5.

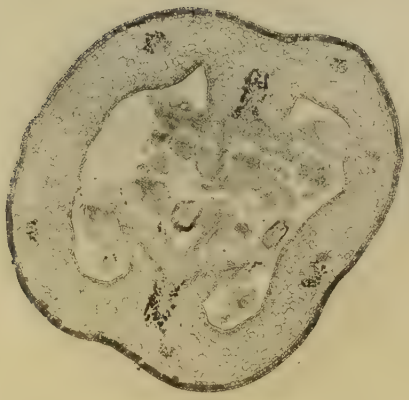

Fig. 6.

Fig. 7.

PROCEEDINGS OF THE ASSOCIATION

Pseudococcus sacchari Ckll. *and its Associates in Madeira.* By Dr Michael Grabham, Quinto do Val, Madeira.

(*Read on* Dec. 7th, 1923.)

The invasion of the Egyptian sugar-cane plantations by the deadly Pseudococcus has led me, in answer to an appeal from the authorities in Cairo, to study and report upon the life-history and associations of the Coccid in Madeira where it is prevalent and unrestrained though negligible in effect on the sugar output owing apparently to the cultivation of a variety of cane overflowing with vitality and equal to a severe strain.

The cultivation of sugar-cane in Madeira seems to have commenced early in the fifteenth century, soon after the sailors of Portugal had stumbled upon the Archipelago in the days of Prince Henry the Navigator. This Prince had an English mother and his life was devoted to the expansion of his country and colonial development.

The cane came from Sicily and after the westward enterprise of Columbus seems soon to have spread from Madeira both to Canary and the West Indies.

Preference was always given to the cultivation of the vine, for it was soon realised that sugar could be grown in any warm country whereas the specific quality of Madeira wine was limited and local.

From time to time a blight or other cause would obliterate the vine, and sugar-cane would become predominant: and again in like manner the cane would periodically fail.

Thus in regard to the vine, the vineyards were completely destroyed in 1852 by the fungoid *Oïlium Tacteri*, and again in 1877 by the underground apterous Aphis *Phylloxera vastatrix*.

But in these days the maintenance and favour of the sugar-cane have been stabilised in Madeira by preferential regulations at Lisbon.

The Bourbon variety of cane had been cultivated in Madeira from time immemorial until the year 1877, when a fungus, probably *Aspergillus fulvus*, was introduced and spreading along over terraces destroyed every cane by intrusion into the stem substance where it converted juice and sugar into a thick mucilaginous nauseous ooze. The fungus itself died with the cane and has not since reappeared.

No attempt was made to replant sugar-cane after that dire destruction and the ground was occupied for several years by the vine, sweet potato and general food produce.

The *Pseudococcus sacchari* was sparsely present before the arrival of the *Aspergillus* fungus and I think was largely responsible for the introduction of the fungus through its many punctures into the substance of the sugar-cane already weak from prolonged cultivation and loss of protective power.

Strenuous efforts were now made to find a cane new to the locality and of greater resisting power, and the Yuba cane from Natal was adopted and has since thriven and yielded well.

The Pseudococcus either survived the Bourbon cane destruction in some manner or was reintroduced. At all events it appeared at once as the Yuba cane spread in the same sparse presence as in the years anterior, to the coming of the *Aspergillus fulvus*.

The Madeira terraces were bare of sugar-cane for quite three years, thus giving ample time for the mealy bug to die out, if not otherwise sheltered.

For a while I felt fairly certain that a *modus vivendi* had been provided by the elegant tomato tree, *Cyphomandra betacea*, and also by the shrubby *Guatamala solanum*, with the result that both these plants of high economic value have practic-

ally disappeared from this island: but my experiments this year go far to acquit the bug of this evil, for though I have transplanted whole colonies of it with the greatest care on a thin paring of cane into the seclusion of banana or strelitzia sheathing, into the fleshy stem of dichorisandra, or the attractive juices of the custard apple, I have in no single instance succeeded in persuading the noxious creature to thrive or even survive away from the sugar-cane, and in all this I am fully confirmed by the experience of Mr Ward, the entomologist of the Egyptian Government.

I am just now watching a badly infested plot from which my men have outrooted the cane and without further preparation planted the sweet potato convolvulus in trenches filled with sugar-cane debris swarming with Pseudococcus. The convolvulus is all rooted and growing rapidly, but though the plant is badly subject to lecanium scale, not a single individual of the sugar mealy bug has appeared. I have found a spray of bitter quassia to be distinctly effective against the bug in an early stage.

The Argentine ant found its way to Madeira 30 years ago when the Yuba cane was already well-established, and the response of the Pseudococcus to the fostering influence of the new pest became at once manifest in the startling increase and activity of the cane parasite. The ground was honeycombed with formicaries among the cane roots, and during the fortnightly irrigation of the cane the legs of my men were black with the myriads of ants seeking this shelter from the running stream of water among their nests.

I may mention, in illustration of the fecundity of the Argentine Iridomermyx, that I had destroyed 40,500 ants from a single lemon tree without making a tangible impression upon their numbers.

The articles of partnership between this ant and the Coccidae I have never mastered, though Madeira abounds with opportunity for study. The ant certainly provokes or augments the flow of honey dew and also ministers obviously to the welfare and overwhelming fertility of the bug. Without the ant the Pseudococcus and its kindred languish and wander disconcerted: and though they can run alone without the ant, they dwindle in numbers and are no longer formidable in their depredations.

The Pseudococcus is now found abundantly in every cane plantation in Madeira: you may look for it with confidence under every leaf sheath which has separated from the growing stem sufficiently for the young migrating female to enter: and a colony at the node where they congregate will number from twenty to several hundreds of individuals in every stage of growth. All of them are females and reproduce parthenogenetically in fabulous numbers, and a full-grown cane will support very many well-established and thriving communities in countless myriads.

The bug feeds on the cane stem itself, attaching itself very rarely to the leaf sheath. From every colony a stream of honey dew laden ants constantly issues, many of them carrying besides any debris or gummy exudation associated with the mealy bug. The parasitic community is hence kept clean and free from the adhesive impediment to stripping described in Egypt.

The sugar mealy bug requires shelter, and hence when the lower cane leaves dry and retreat from the stem the younger members of the community migrate upwards—naked and not ashamed, to find in more succulent regions an opening to a more sheltered and nutritious node.

You may see on any mature cane tiny wandering females searching for the first sign of a new leaf parting from the stem, ready to enter into a hardly perceptible crevice. It is said that the ant itself transplants the young ones, but I have never seen this and the free mobility of the sugar mealy bug is sufficient without such assistance. But the ant follows the bug as soon as there is room enough to pass within the sheath.

A shower of rain will fill every sheath with water and drown every ant which has not had time to escape, but if you tear the leaf sheath asunder you will find every bug alive and undisturbed by its immersion for many days.

The older ones remain below and there secrete more copiously their protecting mealy threads and waxy exudations. But this species is sparing of its cottony secretion even when exposed.

The male Pseudococcus I have only seen once, viz. in March of the present year on an exposed position of the cane stem. He is probably born late in the life of his virgin parent and seems to pass his ephemeral career in the society of mature or even aged associates whose waning fertility may require sexual recuperation.

He is troubled with no alimentary developments, not even a mouth has been given him, and he is thus able to devote undivided attention to the matrimonial attractions of his exacting mates.

Thus infested and ministering to the sustenance of countless parasites and overflowing moreover to the supply of an insatiable stream of ants who night and day ceaselessly milk their plump protégés, the Yube cane in Madeira has survived in unabating vigour quite 30 years, showing even yet no sign of deterioration either in vitality or crystallisable sugar yield. Such a vitality is truly miraculous and inexplicable. No control measures are undertaken or thought of: the cane is cultivated, manured and cut year after year—sometimes for 20 years without replanting—and Pseudococcus and ant are left to work out their own salvation unmolested.

All sorts of supplementary crops are interpolated and gaps in every plantation are filled with Indian corn, beans and sweet potato plants, and all these thrive until choked out of existence by the overwhelming cane growth which, with a girth hardly exceeding that of the *Arundo Donax*, reaches a height of 8–10 feet in rank superiority and in spite of the drain it suffers from myriads of a relentless parasite.

The *Pseudococcus sacchari* has no restraining enemy in Madeira. It is jealously protected and fostered by the Argentine ant as I have stated, and in the presence of so vigilant a participant of its stolen goods no chance is given to any hungry intruder.

My friend, Professor Cockerell of Colorado, an accomplished naturalist and keen observer, speaks of finding signs of a dipterous parasite among the debris of the cane mealy bug, but I have found no trace of such an enemy, neither has any appeared in the badly infested cane specimens which I have sent to Egypt for comparison.

Formerly we had plenty of lady-birds, both of the common European species and locally specialised forms, and yellow patches of their eggs were freely found on any coccus infested twig. But the ant now destroys every such interloper whether coccinella or kindred scymnus. Hence, so long as this pernicious ant exercises a strict supervision, the introduction of an efficient coccinellid, such as a Cryptolaemas, in some form would be an expensive futility.

Mr Ward, in his excellent account of the Pseudococcus in Egypt, regards the rat as a potent enemy to the cane parasite and speaks of holes or windows torn in the leaf sheath in search of the bug. In Madeira this is not so, no such windows appear, neither does the rat, which is here destructive and abundant, molest the bug when freely exposed by the retreating sheath. But the rat, if undisturbed, will scoop out the luscious substance of the lower part of the cane for 2 or 3 feet and thus leave it to perish.

Of possible fungoid enemies I am aware of none exercising any control of economic importance.

The Yellow Aspergillus obliterated the sugar industry, as I have stated, having, as I believe, been introduced into the substance of the cane through the perforations inflicted by the long sucking tubes of the mealy bug. The red fungoid stain I show you seems to be of a kindred nature, but this, though generally prevalent in the leaf sheath, has not been known to penetrate the cane itself. To verify this, I have carefully grafted fungus infected sheathing into the cane substance, and paring the surface of both cane and sheath, I have secured both together without obtaining the faintest staining of the cane tissue. And I know of no other fungus in association with the cane or mealy bug.

We have thus in Madeira a variety of cane which in 30 years has suffered no impairment though beset with exhausting parasites. Our sugar is yielded in full volume and of the attractive quality of the specimen before me.

The object of this paper is to contrast the local conditions at Madeira concerning the sugar-cane and its Pseudococcus parasite with those reported from Egypt, and to elicit what is known of the subject elsewhere.

The Work of the Fisheries Laboratory, Lowestoft[1]. By E. S. Russell, M.A., D.Sc., Director of Fishery Investigations, Ministry of Agriculture and Fisheries.

The laboratory consists of a large detached building on the South Esplanade standing in its own grounds, and directly overlooking the sea. Ample accommodation is provided for the staff and there is plenty of light and air. The laboratory is now reasonably well-equipped with apparatus and there is a useful library which is being gradually improved. Our chief lack at present is a tank-room with a circulation of sea-water. This is a very serious drawback, but it is hoped in the future, when the country is more prosperous and money for research is not so tight, to have a proper series of tanks constructed and housed in a tank-room to be built in the grounds. A number of small table tanks have been provided in one of the basement rooms and an aerating system has been installed, modelled on that in use at some of the Danish laboratories.

Our research ship the *George Bligh* is an ex-Admiralty trawler of the Lord Mersey type, built in 1917. Certain alterations have been made to adapt her for fishery research and accommodation has been provided for the naturalist staff forward, in part of the former fishhold, and a laboratory under the bridge in what used to be the skipper's cabin. Her fishing equipment is, generally speaking, that of an Iceland trawler. She has a 1000 fathom winch enabling her to trawl in 200–300 fathoms. Special equipment includes a steam Lucas sounding machine and a special light winch mounted on a platform in front of the bridge which has proved very useful for working small gear.

Her maiden voyage as a research ship was made in April 1921 to the Lousy Bank, which is a little-known Bank lying between Rockall and Fäeroe. Very bad weather was experienced on this trip, which was unsatisfactory so far as finding fish was concerned, but she behaved extremely well and has proved to be an unusually steady and comfortable ship. A small bank discovered near Rockall has been named George Bligh Bank.

We have at present no small vessel for inshore investigations attached to the laboratory[2] but this lack has been partially remedied by the installation of a motor in one of the ship's lifeboats, this being detached temporarily from the ship, for a day or two at a time, for work in such places as the Wash and the Thames, or along the Suffolk coast.

As regards the kind of work we have been doing and plan to carry on and develop in the future, the main energies of the staff both under the M.B.A. and under the Ministry in London were devoted to the study of the plaice. By 1914 the plaice investigations had been in the main brought to a conclusion; the broad outlines of the natural history of the plaice in the southern North Sea were pretty thoroughly known, and definite proposals for the protection of the plaice, based upon the research work of all the participating countries, had been put forward by the International Council for the Exploration of the Sea. Then came the war, which brought about a very striking and very instructive change in the size and distribution of the plaice in the North Sea. There were more plaice than before, and they were markedly larger and older than the pre-war plaice. The growth-rate had diminished and there was a partial reversion to the state typical of a virgin ground. This interesting development demanded investigation, the more so that certain continental countries revived the proposal for an international size limit. A year's intensive study of the plaice was agreed upon at the I.C. meeting in March 1920 and duly carried out. I do not propose to enter into the methods and results of that year's work, interesting as the results were[3]. Plaice work on a big scale was definitely brought to an end in April 1921, and plans made to begin the study of other fish of equal importance. At the I.C. meeting in July 1921, we undertook to devote special attention to the cod and the herring, and all our work since that date has been concentrated directly or indirectly on cod and herring problems.

[1] Read on Nov. 10th, 1922, revised and brought up to date (Dec. 1923).

[2] One has recently (Jan. 1924) been acquired for work in connection with local by-laws.

[3] See J. O. Borley and others, "The Plaice Fishery and the War," *Fishery Investigations*, Ser. II, vol. v, no. 3, 1923.

The first line of investigation which we started was the quantitative study of the bottom-fauna in relation particularly to the food of cod, haddock and plaice. Many of you are no doubt familiar with the pioneer work of Dr Petersen in the Belts and the Cattegat carried out by means of his bottom sampler, which enabled him to map out the communities of bottom living animals in those waters and to estimate the approximate amount of available fish food per unit of area. We have been successful in applying this method in the open waters of the North Sea.

With the kind assistance of Prof. Hjort, an arrangement was made with Dr Petersen to send his assistant Dr Blegvad to Lowestoft to put us wise to the method of using the bottom sampler and the technique of working up the results. A special cruise was run by the *George Bligh* from Lowestoft to Esbjerg in July 1921, when 20 stations were worked. Dr Blegvad, who took part in this cruise, has written an account of it which has been published by the Zoological Society[1]. The main results of this preliminary survey were to show that the bottom sampler could be used without much difficulty from a biggish ship in open waters, and that several of the communities distinguished by Petersen were to be found in the North Sea—notably the *Venus* community and the *Echinocardium-filiformis* community. Having thus been shown the way, we have since devoted a good deal of time to work with the bottom sampler. It was found best to use a larger form of grab than that generally employed by Petersen, as the additional weight made it delve deeper into the bottom and rendered it less liable to overturn in bad weather when the drift of the ship is apt to make the haul an oblique one. The grab we use has an area of 0·2 metre sq. or roughly 2 sq. ft.

As it was clearly impossible to sample the whole North Sea adequately we concentrated first on an area on the Dogger Bank about 30 miles square, with the idea of working this thoroughly two or three times a year. Casual survey work is done in addition on ordinary cruises with a view to mapping out in a general way the distribution of the communities in the North Sea. The Dogger Bank area was selected for intensive work because of the proposal (which at one time seemed likely to come to fruition) to transplant small plaice on a commercial scale from the Continental inshore waters to the Dogger Bank. It seemed desirable to make some sort of estimate of the amount of plaice food present on the Bank, in order to be sure that there would be sufficient available for the new population.

The results of an intensive survey of the selected area on three cruises carried out in the autumn of 1921 were instructive. The fauna was found to be very patchy. In general it was not particularly rich, but here and there very rich patches of *Mactra* (*Spisula*) *subtruncata* were found. Thus at one station over 200 large specimens were taken in one haul from an area of 2 sq. ft., and at another over 800 small specimens of the same shellfish. As this form is greatly sought after by the plaice the result is an important one from the fishery point of view.

The fauna belonged generally to the *Venus* community with a tendency to Deep *Venus*, but the correspondence with Petersen's communities was not a close one and it may be necessary to found a new community for this Dogger area or perhaps to revise the whole community idea in the light of our recent results.

Among the interesting forms found by the use of the grab were *Amphioxus*, which we found to be common in patches of gravel on the Dogger, and a specimen of *Glossobalanus*[2] taken off the Northumberland coast. It is hoped also by seasonal work to get some useful data as to the rate of growth of the commoner forms on the Bank.

During 1922 we continued the investigation, extending it to the whole Dogger Bank area, on a more intensive scale than before, paying special attention to determining the extent of the rich patches.

A particularly successful cruise was carried out in October when the greater part of the Dogger Bank was surveyed, 150 stations being worked at 5-mile intervals, two hauls at each. The result has enabled us to map out the positions and extent of several large patches both of *Mactra stultorum* and of *Spisula subtruncata*, the patches of the latter being several miles in length and breadth, and showing a remarkable gradua-

[1] *P.Z.S.* 1922, pp. 27–32.

[2] See A. Meek, *Q.J.M.S.* LXVI, 1922, pp. 579–94.

tion from small numbers of these shellfish at the edges to very high numbers at the centre of the patch, the range on a typical patch being from 5 to 8000 per metre square. Much information is being accumulated as to the rate of growth, time of spawning and death-rate of these shellfish. A preliminary report on the results up to February 1923 has been published by Mr F. M. Davis[1] and the work is still going on.

In connection with these investigations a type collection of the North Sea fauna is being formed and contains now 400–500 forms. The stomach contents of many fish, particularly the cod, have been examined.

Another main line of research has been the study of the early stages of the herring. We have broken away from the orthodox plan of concentrating attention on the age and maturity of the adult herring and have given most of our time to a search for the spawning grounds and the larvae and post-larval forms. Surprisingly little is known about the spawning grounds of the herring in the North Sea or about its history during the first two or three years of its life, and we are trying to fill up this gap so far as the English area of investigations is concerned. We began by making a thorough search for herring spawn in certain areas, off the Northumberland coast mainly, and on the west and south edge of the Dogger, where it is known, principally through the landings of spawny haddocks, *i.e.* haddock gorged with herring spawn, that the herring do spawn in August–October. The search for the spawn itself, carried out by all forms of bottom gear, was a complete failure, either because the spawning was unusually restricted in amount or because we failed to find the patches. We found it much more profitable to go for the newly-hatched larvae and the post-larval forms, and with this object in view several lines of stations were worked repeatedly in 1921 and 1922 over the southern North Sea right into the Heligoland Bight, in the Wash, the Thames and the eastern part of the Channel.

Very rich material for study has thus been obtained, the principal gear used being the Petersen young fish trawl.

Though the results have not yet been fully worked up it is already evident that there is a spawning ground off the Lincolnshire coast which no doubt contributes largely to the rich shoals of young herring known to frequent the Wash. Other spawning areas, already partly known, were discovered off the Northumberland coast and on the west edge of the Dogger. Large catches of post-larval herring—up to about 1000 in a half-hour haul of the Petersen trawl—have been made also at certain places in the Southern Bight, and the eastern Channel stations have also proved productive. A concentration in shallow inshore waters appears to take place at quite an early stage in the herring's life-history. It is hoped by continuing this line of investigation to map out the chief spawning areas and trace the passive distribution of the early stages. One significant and rather troublesome fact emerged from a special study carried out in 1921 on the variation in abundance of young herring. The ship was anchored for 24 hours and four sets of hauls by the Petersen trawl were taken at intervals. There was found to be a very big variation in the quantity of post-larval herring caught, a variation due not to vertical movements in the water but apparently to shoaling. This shows that one must be cautious about drawing quantitative conclusions from the catches made of the post-larval herring, even under an inch or so in length.

I come now to an interesting piece of special work on the herring which we carried out in 1921.

As you probably all know, the herring fishery in 1921 on the East Coast of England was a comparative failure. The fish were not found in their usual abundance; they were generally late in arriving at their usual haunts and they were ill-nourished. It had already been shown by the Scottish investigators that an abnormally large influx of Atlantic water had taken place round the north of Scotland into the North Sea in 1920 and 1921 and it was suggested that some connection existed between these abnormal hydrographical conditions and the scarcity of herring. We therefore arranged a cruise in October 1921 through the chief herring areas and across the

[1] *Fishery Investigations*, Ser. II, vol. VI, no. 2, 1923.

North Sea north of the Dogger as far as the Great Fisher Bank. Hydrographical and plankton samples were taken at frequent intervals and the hydrographical results demonstrated quite clearly the presence on the bottom north of the Dogger and also in the gut between the Dogger and the coast of a thick layer of Atlantic water extending farther south than usual[1]. The plankton results at certain stations, particularly between the Dogger and the coast, gave an apt confirmation of this result, for large quantities of the Atlantic pteropod *Limacina lesueuri* occurred together with the more usual *Limacina retroversa*, also a species of *Peraclis* and the Atlantic radiolarian *Phyllostaurus quadrifolius*[2].

Additional evidence was found in the temperature and salinity records from various lightships and steamship routes for 1921 of the encroachment of the Atlantic water[1]. What the relation between this unusual state of the North Sea and the scarcity of herrings is, cannot be stated, but it seems probable that there *is* a relation and, bearing in mind the poor nutritive quality of the fish, it seems likely that this relation is a mediate one through the food supply. In this connection certain results obtained in 1920–1 from a quantitative study of the plankton associated with plaice eggs and larvae in the Southern Bight may be significant. It was found that the plankton was richest, not in the tongue of Atlantic water which pushes up into the North Sea from the Channel during the winter, but at the sides of it, in the coastal water, particularly on the Dutch side[3].

In any case it is obviously important for us to make a close study of the food of the herring and of the origin of that food. A beginning was made with this line of investigation during 1922 and it was actively prosecuted during 1923. Study of the very young stages has yielded results confirmatory of Miss Lebour's work at Plymouth. The very young ones, even before they lose the yolk sac, appear to select their food from the plankton and show a very marked preference for *Pseudocalanus*. Larger ones go for *Temora* and the herring of whitebait size so far as studied seem to specialise on *Eurytemora*. A good deal has also been done on the food of the adult herring. It has proved necessary to get quite fresh stomachs, as digestion appears to be rapid and to continue after the death of the fish, but samples are being obtained for us on drifters by the crews. One sample, taken by the *George Bligh*, had been feeding largely on cod and plaice eggs.

Though we are concentrating rather on the early stages of the herring's life-history the study of age and maturity is by no means being neglected. Many samples of commercial herring have been examined, and uniformity of method has been achieved by sending a member of the staff to Norway to study under Prof. Hjort and Mr Einar Lea.

Before leaving the subject of herring investigations I might mention that a member of the staff made two or three flights in a seaplane off Lowestoft to investigate the possibility of spotting herring shoals from the air. The experiments were carried out in co-operation with the Air Ministry during the height of the herring season. The flights were quite successful and not marked by any untoward incident but the results were definitely negative, as it proved impossible to see the herring owing to the water being thick with suspended sand and mud. Better results have been obtained by the French in the clear waters off the Brittany coast, especially by the use of an airship. Further flights were arranged off the Cornish coast, but were unsuccessful owing to bad weather.

We come now to the cod. A large amount of data is being collected as to the size of the cod taken by the trawl through the agency of a staff of fish measurers who make trips constantly on commercial trawlers and measure large samples of the catch. The plaice and the haddock are also measured, and the haddock measurements are handed over to Scotland under an agreement by which Scotland tackles the haddock while we specialise on cod.

Special work on the cod, apart from the routine fish-measuring and the bottom

[1] See J. R. Lumby, International Council, *Public. de Circonstance*, no. 80, 1923.
[2] See A. C. Hardy, *Pub. de Circ.* no. 78, 1923.
[3] R. E. Savage, *Fishery Investigations*, Ser. II, vol. v, no. 6, 1923.

fauna work, has consisted hitherto principally in a series of marking experiments carried out, first at Flamborough on the Yorkshire coast by a member of the staff working in the local cobles, and later by the research vessel. About 750 cod, mostly large ones, have been marked, and the returns have been good. A large number of cod have been examined on the market at Bridlington and at sea as to their state of maturity, and much work has been done on the food. Statistical studies have also yielded valuable results[1].

I come now to the last main line of work carried on, namely, hydrography. My policy here has been, for reasons which will appear later, to carry out a small regular programme of general hydrography on lines suggested by the Hydrographical Section of the I.C. and to continue in a revised form the system previously inaugurated of obtaining surface observations of temperature and salinity on steamship routes.

The general hydrographical programme which we put into operation at the beginning of 1922 at the instance of the I.C. comprises (1) current observations by means of the Jacobsen meter three times daily at several depths on the Varne and Smith's Knoll Light Vessels, (2) daily observations at the same Light Vessels of surface salinity and temperature. In addition the observations hitherto carried out at the Seven Stone Lt. Vl are being continued as well as those made on the various cross Channel routes and on one route across the North Sea. New lines of steamship observations have been started in the Irish Sea and across the southern part of the North Atlantic. On the other hand, regular sampling from the Research Vessel has for the most part been discontinued, as it has been found that such observations are too sporadic both in space and time to be really valuable. Observations of temperature and salinity at different depths are however made from time to time in direct relation to specific fishery problems. We have arranged also to carry out every year two or more hydrographical cruises along the general line Tyne to Naze of Norway, with the purpose of getting some idea of the spread of Atlantic water from the North, and these cruises have been regularly carried out. Much work is also being done on currents by means of drift bottle experiments.

Having given you an idea of the sort of work we are doing at Lowestoft I propose now to say a little about the guiding principle on which it is all based. This guiding principle is a very simple one. It is that the chief aim of fishery research should be to gain a complete and many-sided knowledge of the natural history of all the fish of economic importance, a knowledge such that rational measures can be taken to conserve and possibly in certain cases to increase the supply available for food. All the different lines of fishery work, as for instance plankton investigations, bottom fauna work, statistics, fish measuring, hydrography, ought to be concentrated on the study of the particular species of fish which is being investigated. The fish ought to be the centre of the investigation and all lines of work should be definitely and closely related to this central problem. Thus investigation of the plankton should not be carried out, by a Fisheries Department at least, primarily for its own sake, but from the point of view of its forming the food at some period or other of the particular species of fish being studied. We should make a special study of those plankton species which are in this way important, investigating their origin, distribution, conditions of development and growth.

In the same way, in studying the bottom fauna, we should concentrate attention on the species which are important as fish food, try to estimate the quantity of this fish food, study it in relation to the stomach contents of the fish and its habits of feeding. It should not be our primary aim, as a Fishery Department, to chart out the distribution of all the bottom living forms, however interesting this would be from a purely zoological point of view. From the study of the fish food whether planktonic or benthonic we should pass outwards to a study of the organisms upon which the fish food feeds, and thence to a study of the physico-chemical conditions influencing production in the sea. Our procedure should be to start from the fish and work outwards to the general conditions rather than the other way round. The fish and its history should be the guiding thread running through all our work and giving it significance and coherence.

[1] See Michael Graham, *Fishery Investigations*, Ser. II, vol. VI, no. 6, 1924.

I am aware that this policy of concentrating everything round the fish and leaving out to some extent the general aspects of oceanography as a whole, has its dangers. It is always unwise to concentrate overmuch in obtaining immediate economic results at the cost perhaps of basing these upon insufficient knowledge of the very complex conditions obtaining in the sea. But I am looking at the question primarily from the point of view of the fishery biologist who is bound to keep economic questions well in the forefront and to try and get results definitely of use to the trade and to the nation.

There are other bodies at work both in this country and in others whose function it is to study oceanographical problems and problems of general biology and physiology from a purely scientific point of view. Witness the many excellent papers produced by the Marine Biological Association in this country and the work which used to be turned out at Naples. All that is available for study and instruction, and to give pointers for economic research.

Then again, I must admit that this intensive scheme of fishery research rests upon the great and valuable work done in the past upon general lines. We know roughly the general distribution of plankton in our seas, the broad outlines of the distribution and variation of temperature and salinity, the main movements of the water, and without this knowledge our specialised fishery work could not be fitted into its proper place in the general science of the sea. I submit, however, that we do know in broad outline what is happening in our waters and that we can accordingly fit our fishery work into the general scheme of things and see it in its proper relations to oceanography as a whole.

Another reason exists—a not unimportant one at the present moment—why fishery work should be concentrated upon its own special problems and our forces not dispersed too widely on general lines. It is that at present the Fisheries Department has not the means—either in material or in personnel—to do oceanographical work on the grand scale.

We have a staff which is just sufficient to carry out the programme laid down on cod and herring and we have only one ship. This ship is fully occupied in the southern half of the North Sea in biological work. We cannot at the same time tackle any elaborate investigations in other waters, notably in the south-west area off the mouth of the Channel so important for the study of mackerel and hake. Accordingly we have to cut our coat according to our cloth and limit ourselves to doing such work as we can really carry out.

REVIEW

British Hymenoptera. By A. S. Buckhurst, A.R.C.S., D.I.C., L. N. Staniland, A.R.C.S., D.I.C. and E. B. Watson, A.R.C.S., D.I.C. With an Introduction by H. M. Lefroy, M.A., Professor of Entomology at the Imperial College of Science. Pp. 48. 8 plates and 16 text-figures. London, Edward Arnold & Co., 1923. Price 9*s.* net.

The writers of this volume collaborated with Prof. Lefroy in his *Manual of Entomology* reviewed in the last number of the *Annals*, and figures from that book are used for the illustration of this, supplemented by sixty-two reproductions of photographs which are, for the most part, excellently clear. The text consists of brief accounts of the tribes and families of the Hymenoptera with special reference to the commoner British species, which the entomological student is likely to meet, as he starts to make a working collection or to observe the ways of living insects. The general introduction to the order lacks any reference to the nature of the jaws, and the treatment of other important structural features, such as the wings, is very meagre. Despite its small extent the book contains useful information, but the combination of wide pages and large type has resulted in a selling-price that seems somewhat excessive for a work of such restricted scope.